*Journal of Bioenergetics and Biomembranes, Vol. 26, No. 4, 1994*

# The Action of Ascorbate in Vesicular Systems

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*Received March 17, 1994*

Many effects of ascorbate center on its interactions with membranes from plant and animal cells. These actions can be studied using vesicles produced from phospholipid components (liposomes), by isolating naturally occurring vesicles, or by purifying particular membranes that form vesicles during the extraction process. Liposomes have provided information concerning the anti- and prooxidant properties of ascorbate and about how the water-soluble vitamin can have effects within the phospholipid bilayer. The involvement of ascorbate in transmembrane electron transport has been characterized in vesicles normally found in certain cells, such as, chromaffin granules, synaptosomes, glyoxisomes, peroxisomes, and clathrin-coated vesicles. Redox activity using reducing power associated with ascorbate/ascorbate free radical (AFR) has been characterized in some of these vesicles and it appears to be mediated by a *b*-type cytochrome. Ascorbate also participates in the reduction of iron within clathrin-coated vesicles. Vesicles appearing during purification of plasma membranes have transmembrane electron transport, oxidoreductase activity with ascorbate/AFR as redox agents, and an ascorbate-reducible *b*-type cytochrome. It is also possible that ascorbate-related redox activity exists at the tonoplast of plant cells.

**KEY WORDS:** Ascorbate; ascorbate free radical; liposomes; membrane vesicles: chromaffin granules; glyoxysomes; plasma membrane redox; clathrin-coated vesicles; *b*-type cytochrome; iron fluxes.

## INTRODUCTION

Ascorbic acid is present in high amounts in both plant and animal cells, and is acknowledged to play important roles in many metabolic events; it also has clinical applications. The action of this vitamin, however, is quite varied, so that after one discovers that ascorbate is involved in a particular process, much work is still needed to determine the exact nature of the interaction. For example, ascorbate may influence enzyme reactions, usually by acting as a reducing agent, and its oxidized forms may be important as electron acceptors (Diliberto *et al.*, 1991). But ascorbate is also involved with nonenzymatic reactions; in this role, it may serve as an antioxidant by scavenging oxygen radicals (Frei *et al.*, 1990), or it may be a prooxidant by reducing iron or copper with the consequent production of $H_2O_2$ (Navas, 1990; Fukuzawa *et al.*, 1993). The effects of ascorbate in delaying tumor onset and growth as well as reducing the toxic side effects of cancer treatments are other important roles for the vitamin (Block, 1993).

Because the effects of ascorbate in biological systems often involves some sort of interaction with cell membranes, one of the more fruitful methods of studying ascorbate action is the use of membrane vesicles. The vesicles may be artificial, phospholipid-containing liposomes, they may form naturally in the cytoplasm, or they may appear during tissue homogenization. Vesicle preparations allow one to define the medium around the vesicles and regulate the contents of ascorbate or other factors inside the vesicle. Problems associated with synthesis and compartmentation are avoided when whole cell or tissue systems are not used, and it is easier to monitor ascorbate oxidation using vesicles; the latter is very important for investigations with a molecule like

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0145-479X/94/0800-0385$07.00/0 

ascorbate which can be oxidized to products with biological activity. In the case of liposomes, one can even define the composition of the vesicle membrane.

The purpose of this short review is to demonstrate how vesicular systems have been used for a better understanding of aspects of ascorbate action on biomembranes. I do not intend to exhaustively review all of the work related to this subject. What follows is just a sampling of recent reports whose findings not only demonstrate what has been learned about ascorbate using vesicular systems, but also suggest some interesting possibilities for future studies.

## RESULTS WITH VESICULAR SYSTEMS

### Liposomes

Because of the value of liposomes for delivery of drugs, enzymes, and genetic material, there have been investigations into the interactions of vesicle contents with the artificial membrane. Of relevance to this review, liposomes were formed in the presence of ascorbic acid oxidase (AAO), since AAO could affect cell ascorbate levels as well as processes dependent on this vitamin (Mossa *et al.*, 1989; Dini *et al.*, 1991). The size of the vesicle formed in the presence of AAO was one-half that of control vesicles and those with BSA. Ascorbic acid was oxidized by the AAO-containing vesicles, indicating that the enzyme was located at the membrane surface. However, an increased activity after detergent treatment, a failure to wash off enzyme activity, and the results of freeze fracture, as well as labeling studies using antibodies to AAO, led to the conclusion that the enzyme spanned the membrane and about 50% of its activity was masked. The partitioning of AAO into the liposomes may account for their smaller size (Dini *et al.*, 1991).

The well-known induction of singlet oxygen by light acting on certain porphyrins can be modeled using liposomes (e.g. Aghion *et al.*, 1992). It was found that hematoporphyrins, pheophytins a, b, and chlorophylls a, b disrupted membranes when light and oxygen were present. The effect was reduced by beta-carotene. Furthermore, the porphoryrins photosensitize the reduction of methyl red by ascorbate (Aghion *et al.*, 1992). In another study of ascorbate's antioxidant properties on liposomes, the antioxidant action of tocopherol was shown to be prolonged by ascorbate. It was proposed that ascorbate reduced tocopherol free radical that resulted when tocopherol scavenged the peroxyl radical of lipids within the membrane (Niki, 1991).

When Fe(II), ascorbate, and a trace of autoxidized PC was added to liposomes composed of egg phosphatidylcholine (PC), the PC of the liposomes was peroxidized; ascorbate oxidation occurred simultaneously (Fukuzawa *et al.*, 1993). This is one example showing that ascorbate cannot be considered solely as an antioxidant. Experiments with a spin probe indicated that the hydrophilic ascorbate–Fe(II) complex was degrading the probe, which partitions to the hydrophobic region of the membrane. After measuring surface charge, the authors suggest that ascorbate–Fe(II) oxidizes polar OOH groups of PC at the membrane surface. The resulting PC–O$^{\bullet}$ penetrates the membrane where it reacts with unsaturated fatty acid moieties of PC. A chain reaction then occurs, producing PC–OOH, which can diffuse to the membrane surface to react with more ascorbate-Fe(II) (Fukuzawa *et al.*, 1993). These results imply that lipid peroxides and iron are necessary for ascorbate-induced lipid peroxidation; thus, antioxidants specific for lipid peroxide and iron chelators may prevent damage in the presence of ascorbate.

### Naturally Occurring Vesicles

Ascorbate appears to play a crucial role in peptide hormone production in chromaffin granules and synaptic vesicles. The chromaffin granule, in particular, has added greatly to our knowledge of the role of ascorbate in cell metabolism. This area has been reviewed recently (Njus and Kelley, 1993), so only the salient features of the system will be presented here.

The chromaffin granule is a secretory vesicle found in adrenal medulla chromaffin cells. Ascorbate plays a crucial role in these vesicles for the synthesis of epinephrine and norepinephrine. The enzyme, dopamine-beta-monooxygenase, located inside the vesicle, hydroxylates dopamine to form norepinephrine after being reduced directly by electrons from ascorbate (Dharimal *et al.*, 1991a). The semidehydroascorbate or ascorbate free radical (AFR), resulting after electron donation to the monooxygenase, is reduced back to ascorbate by an electron from a *b*-type cytochrome in the vesicle membrane. This cytochrome, *b*-561, is in turn reduced by cytosolic ascorbate (Jalukar *et al.*, 1991; Dhariwal *et al.*, 1991b).

The cytochrome *b*-561 that mediates transmembrane redox activity in the vesicle membrane has a midpoint reduction potential of +140 mV and is reduced by dithionite as well as by ascorbate, but it is not reduced by pyridine nucleotides, glutathione, durohydroquinone, or ferrocyanide. The gene for bovine cytochrome *b*-561 has been cloned and sequenced (Perin *et al.*, 1988), and hydropathy plots of its deduced amino acid sequence show six transmembrane domains (Kent and Fleming, 1990).

Chromaffin vesicle ghosts were used to determine if the transmembrane electron transport system could reduce ascorbate-dependent enzymes other than dopamine-beta-hydroxylase. Harnadek *et al.* (1992) entrapped horseradish peroxidase in chromaffin vesicle ghosts with or without ascorbate and measured the oxidation state of cytochrome *b*-561 by dual wavelength spectrophotometry. When $H_2O_2$ was added, a concentration-dependent, partial oxidation of the cytochrome was recorded. These data were interpreted as indicating that ascorbate was oxidized to AFR during the peroxidation of $H_2O_2$ to $H_2O$. The cytochrome *b*-561 was partially oxidized because electrons were transferred from the membrane by this cytochrome to reduce the AFR as it formed. Cytochrome *b*-561 oxidation in the presence of $H_2O_2$ was also accelerated by ATP. This suggests that pH and a potential gradient generated by the $H^+$-ATPase in the ghost membrane plays a role in electron transport from cytochrome *b*-561 (Harnadek *et al.*, 1992).

Thus, the chromaffin granule provides a model system, not only to study the interaction of ascorbate with a monooxygenase, but also to characterize the transport of electrons across a membrane from a reduced to an oxidized form of ascorbate. The facility of manipulating chromaffin granules has also led to information concerning the nature of the redox process, in particular, that H is the species being donated (Njus and Kelley, 1993). The characterization of chromaffin vesicle components and transport properties may also have important implications for vesicles such as synaptosomes (e.g., Eipper *et al.*, 1992).

A rather different role for ascorbate in synaptosomes and some other membrane vesicles from rat brain was suggested by measurements of $Na^+/Ca^{2+}$ exchange (Matsuda *et al.*, 1990). Ascorbate at concentrations normally found in brain cells inhibited the $Na^+$-energized $Ca^{2+}$uptake noncompetitively after a 60-min preincubation. The inhibition did not seem to involve lipid peroxidation or effects on SH groups. Approximately 90% of the added ascorbate remained in its reduced form after the inhibition was evident, so the authors suggest that oxidation of the vitamin is not required for its inhibitory action. Any neurophysiological role for ascorbate involving $Na^+/Ca^{2+}$ transport in these vesicles is unknown in light of the long incubation period before the vitamin is maximally inhibitory and the failure to reverse the inhibition once it has become established (Matsuda *et al.*, 1990).

Other naturally occurring vesicles with transmembrane electron transport properties are the glyoxysomes found in many lipid-containing plant cells. These organelles have a variety of enzymes that serve to conserve the energy in fatty acids by producing NADH and the carbon skeletons for gluconeogenesis. The NADH does not permeate the glyoxysomal membrane, however, and evidence has been presented that an NADH dehydrogenase complex exists in the membrane to oxidize NADH and to transfer reducing equivalents to a cytosolic acceptor (Luster *et al.*, 1988).

This acceptor may be AFR, since NADH : AFR oxidoreductase activity was found associated with glyoxysome membranes. The activity has some similarities to an NADH : ferricyanide reductase in the same membrane (Bowditch and Donaldson, 1990) and may be related to that detected in membranes of chromaffin granules. In contrast to chromaffin granules that transport electrons from ascorbate into the vesicle to reduce AFR, the glyoxysome is postulated to transport reducing equivalents from within the vesicle to AFR in the cytosol.

Peroxisomes are present in animal and plant cells, and also contain enzymes for carrying out oxidative reactions. Membranes purified from peroxisomes of potato tubers were found to demonstrate both NADH : ferricyanide and NADH : cytochrome *c* reductase activity (Struglics *et al.*, 1993). There was cross-reactivity to antibodies raised against NADH : ferricyanide oxidoreductase from the ER, and spectrophotometric evidence suggested the presence of a *b*-type cytochrome (Struglics *et al.*, 1993).

Unlike the case in glyoxysomes discussed above, the role of a peroxisomal membrane redox chain remains unknown, but the presence of a *b*-type cytochrome and the relationship of peroxisomes to glyoxysomes makes it possible that an ascorbate/AFR redox system exists in the peroxisome as well. It would be interesting to compare amino acid

sequences and physical parameters of the *b*-type cytochromes in glyoxysomes and peroxisomes with the cytochrome in chromaffin granules and see if transmembrane electron transport can occur in the presence of ascorbate and/or AFR.

Another example of naturally occurring vesicles with membrane-bound electron transport activity are clathrin-coated vesicles from rat liver (Morré *et al.*, 1985). Several redox activities were measured, among them an NADH : AFR reductase activity. Addition of NADH + AFR to the vesicles led to generation of a membrane potential as measured by accumulation of carbocyanine dye. Neutral red accumulation, indicative of vesicle acidification, occurred in the presence of ATP and NADH and was inhibited by monensin as was the NADH : AFR reductase activity. While these data are by no means definitive, the possibility is established that a transmembrane redox system exists in coated vesicles and that the transport of electrons may serve to reduce internal stores of AFR (Morré *et al.*, 1985)

Iron uptake into animal cells involves the enclosure of an Fe(III)–transferrin complex into a clathrin-coated, endocytic vesicle followed later by a controlled release to the cytosol. After losing the clathrin coat, the vesicle becomes acidified and Fe(III) is dissociated from the transferrin (Watkins *et al.*, 1991). However, before Fe(III) is released to the cytosol, other steps must occur (Nuñez *et al.*, 1990).

To study the processes occurring after vesicle acidification, Watkins *et al.* (1992) used acidified endocytic vesicles. They found that reduction of the Fe(III) was necessary for Fe release. Since this reduction took place within the vesicle, a transmembrane oxidoreductase was postulated. A model was presented in which transport of ascorbate into the vesicle was an important component of the reduction process (Watkins, *et al.*, 1992). The exact nature of the electron transport process and the role of ascorbate remain to be explained. Ascorbate may also have effects on iron uptake into lysomes (Hoffman *et al.*, 1991).

Ascorbate is postulated to have a role in the formation of vesicles as well. The hydroxylation of proline leading to collagen synthesis is a well-known action of this vitamin. However, the production of collagen also depends on the coordinated deposition of alkaline phosphatase and $Ca^{2+}$. This deposition is dependent on matrix vesicle formation, and ascorbate was found to stimulate the release of alkaline phosphatase-rich vesicles from cultured chicken growth plate chondrocytes to the medium (Wu *et al.*, 1989). The mechanism of ascorbate action is not clear, but this exemplifies another sort of interaction of ascorbate with naturally forming vesicles.

### Vesicles Formed during Extraction

Membranes often form vesicles following tissue or cell disruption, and the vesicles will entrap the homogenization medium. It was thus possible to purify plasma membrane vesicles from soybean seedlings so that they contained 10 mM ascorbate and AFR, the latter postulated to be produced by ascorbate oxidase associated with the vesicle (Morré *et al.*, 1986). This preparation oxidized added NADH, suggesting that electrons were being transported into the vesicle from NADH to AFR.

Plasma membrane-enriched fractions from radish seedlings and cotton roots generated a membrane potential, inside positive, when the vesicles, loaded with a homogenization medium containing 100 mM ascorbate, were suspended in ferricyanide (Hassidim *et al.*, 1987). These data indicated that electrons were being transported across the membrane to ferricyanide and the $H^+$ trapped within the vesicle were responsible for membrane polarization. This interpretation received further support when it was shown that methylamine, a probe that accumulates in acid compartments, was found in higher concentrations in ascorbate-loaded vesicles in the presence of external ferricyanide (Chosek *et al.*, 1991).

Similar results were obtained with plasma membrane vesicles from bean hypocotyls that were largely right-side out (Asard *et al.*, 1992). In this case, transmembrane electron transport from ascorbate in the lumen was measured directly as a reduction of exogenous ferricyanide. Plasma membrane-enriched vesicles of HL-60 cells with NADH in the lumen were shown to drive reduction of external AFR or ferricyanide (Alcain *et al.*, 1991). Unlike the results reported with vesicles from soybean plasma membranes (Morré *et al.*, 1986), however, no effect of exogenous NADH on transmembrane electron transport has been observed (e.g., Hassidim *et al.*, 1987).

Other methods have been employed that emphasize the utility of plasma membrane vesicles as a model system to study transmembrane redox activity. Böttger (1989) showed that NADH introduced into vesicles of soybean hypocotyls by electroporation became oxidized when ferricyanide was added to the

external medium. Using a somewhat different approach, plasma membrane vesicles from sugarbeet mesophyll were loaded with $NAD^+$ and alcohol dehydrogenase (Askerlund and Larsson, 1991). Additions of ethanol caused NADH production in the lumen, and, under these conditions, reduction of the nonpermeating reagent, DCIP-sulfonate, was observed. This effect was stimulated by valinomycin, but reduction of cytochrome *c* or ferric citrate was not detected. However, when the vesicles were loaded with ascorbate, reduction of both DCIP-sulfonate and ferric citrate were now recorded, but the stimulation by valinomycin was much less (Askerlund and Larsson, 1991).

The observations cited above, that entrapped ascorbate can reduce acceptors outside plasma membrane vesicles, lead to the prediction that ascorbate and/or AFR oxidoreductase activity resides in the plasma membrane. Indeed, NADH:AFR oxidoreductase activity has been detected; this activity could be purified away from NADH:ferricyanide and NADH:quinone oxidoreductases, and it had other parameters to distinguish it from the latter two enzyme activities (Luster and Buckhout, 1988; Luster and Buckhout, 1989). Vesicles of rat liver plasma membrane (Navas *et al.*, 1988) and HL-60 cells (Navas *et al.*, 1992) also demonstrate NADH:AFR oxidoreductase activity that may play a role in transmembrane electron transport. Cell surface glycoconjugates (Navas *et al.*, 1988) and a balance of SH/S–S groups (Villalba *et al.*, 1993) were suggested as a means of regulating enzyme activity.

It remains to be shown whether the NADH:AFR oxidoreductase activity characterized using biochemical methods plays a role in transmembrane electron transport. These observations do strengthen the argument, however, that redox systems with different electron acceptors and donors exist at the plasma membrane. One redox system, using NADH as the reductant, transfers electrons to a membrane-bound intermediate that can only be oxidized by DCIP-sulfonate. Another redox system oxidizes ascorbate and transfers electrons to an intermediate in the membrane that can be oxidized by a wider range of reagents, i.e., DCIP-sulfonate or ferric citrate (Askerlund and Larsson, 1991). The latter system may resemble that of chromaffin granules.

Since there is transport of electrons involving ascorbate/AFR as redox agents in plasma membrane vesicles, one might also suspect that a specific *b*-type cytochrome exists in the plasma membrane as it does in chromaffin granules. Careful studies of oxidized versus reduced absorption spectra of plasma membrane vesicle preparations (Asard *et al.*, 1989; Askerlund *et al.*, 1989) have indicated that, indeed, several *b*-type cytochromes are present. The maximum alpha band absorbance of these cytochromes is from 558.8 to 562.5 nm. It appears that 60 to 80% of the cytochrome is reduced in vitro by ascorbate within the vesicle. This ascorbate-reducible cytochrome has an alpha band absorbance at 561 nm, and a redox potential between +120 to +160 mV (Asard *et al.*, 1989; Askerlund *et al.*, 1989).

When plasma membrane vesicles of bean are isolated so they contain ascorbate and they are resuspended in ascorbate plus ascorbate oxidase, an immediate oxidation of the cytochrome *b*-561 occurs, followed by a slower reduction; the kinetics of AFR production from exogenous ascorbate plus ascorbate oxidase parallel the oxidation of the cytochrome (Horemans *et al.*, 1994). These data indicate that AFR can oxidize cytochrome *b*-561; the subsequent reduction of the cytochrome is by electrons from ascorbate within the vesicle. It is possible, then, that the cytochrome *b*-561 detected in vesicles of the plant plasma membrane is a component of an ascorbate-reducible, AFR-oxidizable redox system that transfers electrons across the membrane.

Taken together, there appears to be a striking parallel between plant plasma membrane vesicles and intracellular vesicles like chromaffin granules. Both systems have the capability of transporting electrons across a membrane using ascorbate as an oxidant and both have *b*-type cytochromes with similar absorption spectra and redox potentials. Since the cytochrome *b*-561 in plant plasma membrane is not autoxidizable (Horemans *et al.*, 1994), the natural electron acceptor for transmembrane electron transport may be AFR as it seems to be in chromaffin granules or it may be an iron chelate. In chromaffin granules, however, the pH gradient and membrane potential results in an energy gradient favouring transport of electrons to the inside of the vesicle (Njus and Kelley, 1993). In the plant cell, the lower pH of the apoplast and a membrane polarization, inside negative, favors electron transport toward the outside of the membrane, a situation mimicked by the vesicle preparations, which are prepared in the right-side out configuration.

It is interesting to speculate about the role of a transmembrane redox system located at the surface of

plant cells that involves ascorbate and/or AFR. For example, solutions of ascorbate and dehydroascorbate (resulting in the production of small amounts of AFR) cause acidification of the medium, hyperpolarize the membrane (González-Reyes *et al.*, 1992), and increase the size of onion root cells (Hidalgo *et al.*, 1991). If the electron acceptor is oxygen, a form of active oxygen may be produced and this could be involved indirectly in aspects of cell wall metabolism (Bradley *et al.*, 1992); more directly, the active oxygen may provide a defense against infection (Apostol *et al.*, 1989; Schwacke and Hager, 1992).

The latter suggestion is patterned after redox activity in human neutrophils, which also involves a *b*-type cytochrome. But compared to the cytochrome *b*-561 of the plasma membrane, the spectral properties, the putative electron donor and acceptor, and the redox potential are all different (Asard *et al.*, 1989; Askerlund *et al.*, 1989; Morel *et al.*, 1991; Segal and Abo, 1993). Furthermore, antibodies to neutrophil cytochrome do not cross-react with protein at the plasma membrane (H. Asard, personal communication). NADH oxidase activity that may have similarities to neutrophil oxidase activity has been detected in plant plasma membrane vesicles (Brightman and Morré, 1990), but no specific cytochrome has been identified as an intermediate, and there appears to be no role for ascorbate/AFR.

The ascorbate-reducible cytochrome detected in plasma membrane vesicles may be important for blue light effects in plants. It is known, for example, that a blue light-induced absorbance change (LIAC) can be detected in plasma membrane vesicles; the LIAC implicates a *b*-type cytochrome and it is thought that this represents the same cytochrome that is reduced by ascorbate (Asard *et al.*, 1989; Askerlund *et al.*, 1989). The action spectrum for cytochrome reduction implicates a flavin component and is not unlike that recorded for many physiological responses to blue light. But, the fact that EDTA is the only known electron donor for the LIAC response and that no terminal acceptor has as yet been identified, mitigates the importance of the LIAC (Rubinstein and Stern, 1991). The light-induced redox system, LIAC, is clearly localized to the plasma membrane, and there is evidence for an ascorbate-reducible *b*-type cytochrome at the same location, but there is no evidence that this cytochrome is also involved with blue light-induced events. It is of interest that redox activity has recently been implicated in a phosphorylation reaction that is dependent on blue light (Hager *et al.*, 1993).

"Microsomal" preparations from animals and plants are often composed, at least in part. of vesicles of ER. Redox activity has been found on ER vesicles and an important component is the *b*-type cytochrome, P-450, which is involved with hydroxylation reactions leading to effects on metabolism and detoxification (e.g., Donaldson and Luster, 1991). Cytochrome $b_5$, with antigenic properties similar to NADH : ferricyanide oxidoreductase at the plasma membrane, has also been localized to the ER (Askerlund *et al.*, 1991). Both redox systems use NAD(P)H as an electron donor, but there appears to be no role for ascorbate/AFR (Luster *et al.*, 1988).

Another membrane system that has been purified from yeast and plants is composed of vesicles from the tonoplast. This membrane encloses the vacuole and has many transport systems associated with it, including ATP- and $PP_i$-energized $H^+$ pumps (e.g., Wink, 1993), but redox systems have not been investigated in detail. We have data (B. Rubinstein and L. Taiz, unpublished) demonstrating the presence of NADH : ferricyanide and NADH : cytochrome *c* oxidoreductase activities in purified preparations of yeast and citrus tonoplast vesicles. Furthermore, a *b*-type cytochrome with an alpha band at 561 nm has been identified in the yeast tonoplast preparation (R. Bogolmoni, B. Rubinstein, and L. Taiz, unpublished).

It has not yet been tested whether ascorbate and/or AFR are involved in transmembrane electron transport at the tonoplast. Such a redox system would not be unexpected, however, because of certain similarities to chromaffin granules. The $H^+$-ATPases of both membranes are of the *v*-type (Taiz *et al.*, 1989), the *b*-type cytochromes have similar oxidized vs reduced absorption spectra, and the low pH and membrane potential, inside positive, of both organelles would favor electron transport from ascorbate in the cytosol to AFR in the lumen.

What is more, there are metabolic processes associated with the vacuole that require redox activity. One example is the reduction of rutin by ascorbate after this flavonoid glycoside has been oxidized by UV light or $H_2O_2$ (Takahama, 1986; Takahama and Egashira, 1991). Another example of a requirement for redox activity at the tonoplast is the regulation of ACC oxidase, an enzyme localized to the vacuole, that catalyzes the last step in the production of the hormone, ethylene (Kende, 1993). ACC oxidase is activated in vitro by ascorbate and Fe(II) (Tang

*et al.*, 1993). And, finally, a parallel between tonoplast reactions involving ascorbate and iron fluxes in coated vesicles could be inferred because iron is stored in the vacuole; preliminary results with yeast suggest that mobilization of iron from the vacuole involves a reduction step (Raguzzi *et al.*, 1988).

## SUMMARY AND PROSPECTS

Information has been presented showing that many important actions of ascorbate on biomembranes can be investigated using vesicular systems. The vesicles could be artificially synthesized liposomes, or purified vesicles that are formed naturally by cells, or vesicles of particular membranes that result from cell homogenization.

— Liposomes allow the investigation of the antioxidant and prooxidant activities of ascorbate as well as providing a matrix for reconstitution studies.

— Naturally occurring vesicles have yielded data concerning the role of ascorbate in enzyme reduction leading to hormone synthesis, transmembrane transport of electrons, and identification of the *b*-type cytochrome that mediates this transport. Clathrin-coated vesicles are being used to understand the role of ascorbate in iron reduction and iron transport.

— The use of vesicles resulting from cell homogenization has led to a better understanding of redox activity, particularly at the plasma membrane. Some of these redox processes involve transmembrane electron transport from NAD(P)H to AFR, purification of ascorbate/AFR oxidoreductase activities, and identification of ascorbate-reducible *b*-type cytochromes.

Work in all of these areas continues. Artificial membranes, as well as less defined vesicular systems from plants and animals are being used to analyze the anti- and prooxidant capabilities of ascorbate—both enzymatic and nonenzymatic activities. This may lead to drugs that have clinical applications and to bioengineered animals or crop plants that are better suited to withstand oxidative stresses.

Now that components of transmembrane electron transport systems are becoming identified and their genes cloned, it may be possible to insert these components into liposomes to better study their behavior. Furthermore, vesicles from cellular membranes may show a link between redox reactions involving ascorbate/AFR and various dioxygenase reactions (Prescott, 1993), as already shown for monooxygenase reactions. Another worthwhile area for further investigations is the tonoplast of yeast and higher plants that can be isolated intact or as vesicles. This organelle may have a membrane-bound redox system to maintain reduced ascorbate within the vacuole. The ascorbate could be involved in synthesis of the plant hormone ethylene and in reducing any substances with antioxidant activities.

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