Possibility of Antioxidation Vitamin Derivative Capsules

Shinobu Ito

ITO Co. Lto. Tokyo, Japan

Introduction

Vitamins are classified into two general groups of water-soluble vitamins and lipid-soluble vitamins (table 1). On the other hand, derivatives of vitamins, which are recently produced, are "amphipathic" or soluble to both water and oil. However, ascorbyl palmitate, which is one of amphipathic vitamin derivatives, has been used only as an additive for antioxidizing lipids in food, feed, and cosmetics because its solubility to oil is not as prominent as that to water. A purpose of modifying vitamin derivatives with hydrophilic groups and lipid-base ester is to improve the stability in and solubility to solvents. However, few studies have been conducted on using the amphipathy and surface activity of modified vitamin derivatives compared to those on modification of amino acids and polysaccharides with lipid-base ester. DDS nano capsule for ascorbyl 2-phosphate 6-palmitate (APP), sodium tocopheryl phosphate (TPNa) (figure 1), and ascorbyl tocopheryl maleate (CME) were recently developed by ITO Co., Ltd. These derivatives of vitamins have relatively large surface activities and are accelerating studies on clathrates that use amphipathic vitamin antioxidants.

APP is a water-soluble derivative of ascorbic acid that is little soluble to oil but has a surface activity and can be used as an emulsifier and nanocapsule clathrate. When a water solution

Table 1 Major vitamin C and derivatives registered in the list of ingredients for cosmetics in Japan.

(lipid-soluble derivatives)	(Water-soluble derivatives)
Ascorbyl 6-stearate	Disodium L-ascorbic acid sulfate
Ascorbyl 6-palmitate	Sodium ascorbate
Ascorbyl 2,6-dipalmitate	Magnesium L-ascorbyl 2-phosphate (quasi drug ingredient)
Ascorbyl 2,3,5,6-tetraisopalmitate	Sodium L-ascorbyl 2-phosphate (quasi drug ingredient)
Potassium ascorbyl tocopheryl phosphate	Ascorbic acid 2-glucoside (quasi drug ingredient)

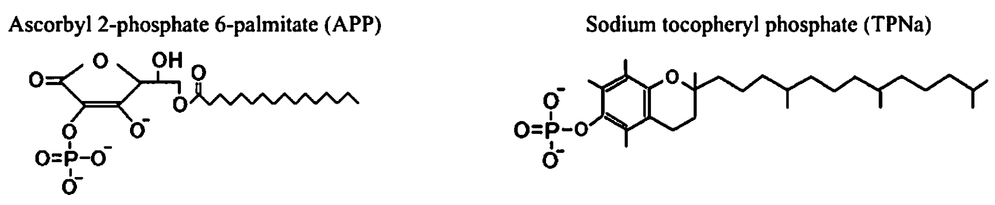

Figure 1

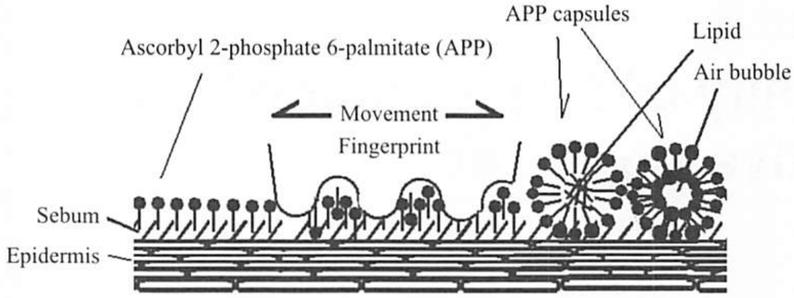

Figure 2 Self-emulsification of ascorbyl 2-phosphate 6-palmitate (APP) on the skin

containing 1% APP is applied on the skin and rubbed in, the transparent solution becomes cloudy and turns into cream-like gel on the skin (self-emulsification). When further rubbed in, it becomes transparent again and agrees to the skin like ordinary creams. This is likely because the APP solution incorporates squalane, cholesterol and other fatty materials present on the skin and forms micelles by self emulsification, trapping in air and forming minute bubbles (figure 2). APP solution becomes "emulsified cream" by mechanical action between the sulcus cutis of the finger and the skin.

Derivative of vitamin C with surface activity

Some emulsion products produced using the surface activity of APP are shown in figure 3. Surfactants are used in many articles we daily use, including cosmetics, foods, medical supplies and paint, but chemical surfactants are prone to irritating the skin. The skin is known to have a structure consisting of piled up keratinized cells with layers of lipids and water. Because the cell membrane consists of two layers of phospholipids, amphipathic materials are easy to penetrate into the skin. Amphipathic materials that are toxic to the cell (destroy the cell membrane, *etc.*) have a tendency of skin irritation. Because many consumers believe "chemical surfactants are irritating", they have a trend to use natural surfactants as much as possible. APP is an amino acid system that transforms itself entirely into vitamin C, phosphoric acid and palmitic acid

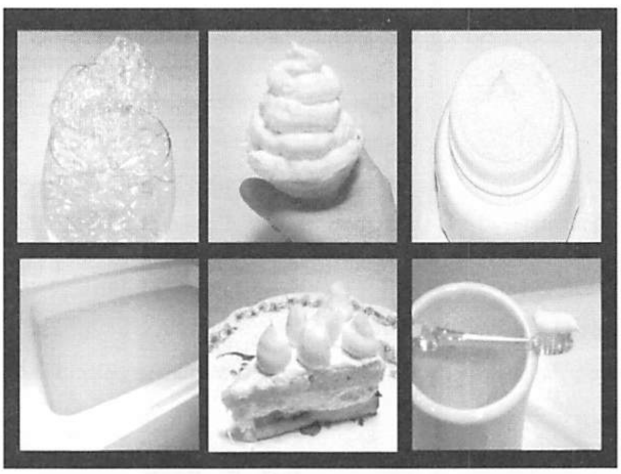

Figure 3 Examples of articles manufactured using APP as surfactant on a trial basis
From the left top, soap bubbles in a glass (bubbles), soft ice cream, cosmetics (whitening cream), bath salt, cream on the cake, and toothpaste

within the body. In our laboratory, we experimentally produced a skin-care cream, bath salt, toothpaste, shampoo, hair mousse, kitchen detergent, laundry detergent, cake, ice cream and soap bubbles using the surface activity of APP (Some are shown in figure 3). These products are likely to be welcomed by consumers who care about the safety of surfactants.

Many of the products, in which APP was used, received good scores on the feeling of use, such as "soft" and "mild". The reason for being "mild" is not clear, but it was possibly because the active oxygen elimination activity of the vitamin C surfactant may have influenced the taste and tactual sense. Factors that trigger the generation of active oxygen are believed to exist in quantity in daily life, such as in tap water, solar radiation, photocatalysis, medical supplies, pesticides, building materials, paint and adhesives. Vitamin C surfactants improve the feeling of use possibly because their strong oxygen elimination activity reduces active oxygen in the living environment. We have also developed applications other than those shown in figure 3, such as for dissolution aids of medical supplies, paint, photographic paper, fiber, gauze and surgical thread, and started joint studies for some products. Although APP is likely promising in various fields, the use is legally accepted only for cosmetics, and legal registration is needed for commercial use of APP in foods, feeds, and medicines.

Derivative of water-soluble vitamin E with an ability to form transparent gel: TPNa

Sodium tocopheryl phosphate (TPNa) is water-soluble white crystals and forms gel when dissolved in water at a concentration of 1 % to 2 %. The gel gives a stretching feeling like polysaccharides, forms a strong water-holding barrier on the skin, and becomes a transparent viscous fluid of a good and non-sticky touch. Furthermore, TPNa is attracting attention for it controls hyperoxidation of lipids, prevents roughness of the skin caused surfactants (SDS), kills acne bacteria, controls the expression of the annexin gene under the exposure to UV-B, mitigates photo aging, and reduces the toxic effects of drugs. In the field of cosmetics in Japan, it is registered as an effective ingredient for preventing roughness of the skin and is used in quasi-drugs category (functional cosmetics). Like APP, TPNa can be used as an emulsion film agent, and it is used for coating capsules, which highly increases the stability of lipids against oxidation because TPNa has a vitamin E structure in itself. It is also effective for controlling natural pigments from fading, such as astaxanthin.

Ascorbyl-2-phosphate (AP)

APP is a derivative of ascorbyl-2-phosphate with an additional fatty acid at its sixth place. It is easily converted into ascorbyl-2-phosphate (AP) in the living body by the activity of esterase. APP can be used as a supplier of ascorbyl phosphate, which is widely used today and has been reported to express the majority of the efficacies of AP[1]. Studies on AP are briefly described below[2,3].

AP is more stable than ascorbic acid. AP is hydrolyzed into ascorbic acid by the activity of alkaline phosphatase, which exists in the serum and near the stratum basale epidermidis. Because AP is gradually converted into ascorbic acid in the stratum basale epidermidis, it does not cause sudden increases in the concentration of free ascorbic acid on and in the skin. At high concentrations, free ascorbic acid is toxic because it turns into ascorbate radicals by UV-B. AP controls the cytotoxicity of ultraviolet ray B to human keratinocytes (HaCaT) but has been reported to not control free ascorbic acid[4]. AP has been used as a so-called skin lightning ingredient for preventing stains and freckles caused by sunburn since the 1970's[5], and has been reported to prevent and improve chromatosis such as chloasma[4,5], control disorders caused by UV irradiation, and promote collagen synthesis[7]. AP is produced in stable salts of Mg and Na rather than in a free form. Its Na salt is an anion in water solutions and penetrates into the depth of the skin easily by iontophoresis, and is particularly effective against chloasma[10] and chromatosis[8,9].

The effects of AP on the skin tissue include inhibition of melanin synthesis by inhibition of the tyrosinase activity[10] and those against UV irradiation disorders, such as canceling activated oxygen[11], controlling oxidation of lipids[12] and preventing apoptosis in the skin[11]. Other physiological effects have also been reported: promoting collagen synthesis in skin fibroblast[14], promoting basal membrane formation and cytodifferentiation[15], controlling the shortening of telomere[16], inducing the formation of tissues and organelles[17], activating the synthesis of collagen types Ⅰ, Ⅲ, and Ⅳ, promoting the construction of extracellular matrics such as sulfated proteoglycan and glycosaminoglycan[18], and preventing damages to DNAs[19]. Recently, it has been reported to improve acnes[20], reduce sebum secretion, and reduce the size of pores[21]. Many applicational studies have also been conducted in the fields of feed, food and medical supplies.

The anti-oxidants like AP, TPNa and Fullerene were effective as an inhibiter of these abnormal behaviors which the limited assembly of stimulant and opioid like methamphetamines was induced. It is interesting that the extreme increase of AA* that was thought iron protein relation was also found in striatum tissue where oxidation is renewed by administration of stimulant drug.

High concentration of the ascorbic acid in the cell

APP is characterized by high absorbency into the living body and expression of high ascorbic acid activity. The principal effects of APP are described below. Kato *et al.* compared the incorporation of APP into normal human skin keratinized cells HaCaT with that of AP, which is a conventional derivative of ascorbic acid, by culturing the cells in media containing various concentrations of APP and AP [26]. The method involved transferring a certain number of cultured HaCaT cells to a medium containing 0.1 mM of either AP or APP, collecting the cells at 3, 6, and 18 hours of culture, rinsing and crushing the cells, and measuring the content of ascorbic acid. The measurement showed that the amount of ascorbic acid accumulated in the cells was larger when cultured with APP than with AP and the addition of the hydrophobic group increased the intake markedly. Miwa *et al.* reported in detail that AP and APP were both very effective provitamin C to be applied on the skin[27,28]. They showed in an APP incorporation experiment using the actual human skin tissue, that the incorporation of APP into the skin tissue, particularly into the dermis, was especially high among various derivatives of ascorbic acid. These and other many reports have suggested that APP added to a culture medium at a low concentration is efficiently incorporated into cells and releases ascorbic acid within the cells and shows antioxidation activities.

Promotion of collagen synthesis and inhibition of tyrosinase activity by APP

Kato *et al.* reported that APP highly promoted collagen synthesis and caused large accumulation of vitamin C, which inhibits tyrosinase activity, in the cells and thus had a skin lightning effect[20]. They reported that human fibroblast cells cultured in media containing various concentrations of APP or AP ranging 1−10 μM for a period of 72 hours showed significant promotion of collagen synthesis even at low APP concentrations. APP has been reported to inhibit the activity of tyrosinase in a concentration-depending manner in human melanoma, mouse melanoma, and mushroom cells.

Production of micro and nano capsules (ITO-Nano DDS) using APP

APP is amphipathic and forms a liquid crystal structure when it is used as a surfactant together with lipids, enabling capsules consisting of APP film and containing lipids to be prepared. APP gel in a liquid crystal state has a multilayer structure and thus can incorporate water-soluble materials in its aqueous layer (figure 4).

Multilayered liquid crystal structure of lipid-containing APP capsules of the self-emulsification type (ITO-NanoDDS Capsule)

The APP capsules prepared as described above transform into self-emulsifying gel, with which minute emulsion dispersions can be prepared just by adding water. The resultant suspension of self-emulsified dispersions is anionic because APP forms the capsule film; and the suspension shows electrophoresis when voltage is applied.

Electrophoresis of APP capsules

APP capsules (ITO-Nano DDS capsule) are negatively charged and minute and thus shows electrophoresis when voltage is applied. figure 5 (1) shows microscope image of ITO-Nano DDS capsule which was dissolved in between micro electrodes before voltage application. When voltage was applied, the ITO-Nano DDS capsule particles moved to the direction of the arrow direction away from the (−) pole (2) with the black zone stretching from the left to right. When the current was reversed, the ITO-Nano DDS capsule particles moved back, causing the black zone to shrink (3). The semicircle objects at the right and left ends are the microelectrodes.

Using this phenomenon, it is possible to introduce non-electrically charged materials, such as lipids, and water-soluble materials, such as peptides, carbohydrates and antibodies using iontophoresis. We have already succeeded in producing self-emulsifying and lipid-containing APP capsules that enclosed CoQ_{10}, astaxanthine, VCIP (ascorbyl tetraisopalmitate), and fullerene (table 2). These APP capsules showed improved stability of APP from that of APP

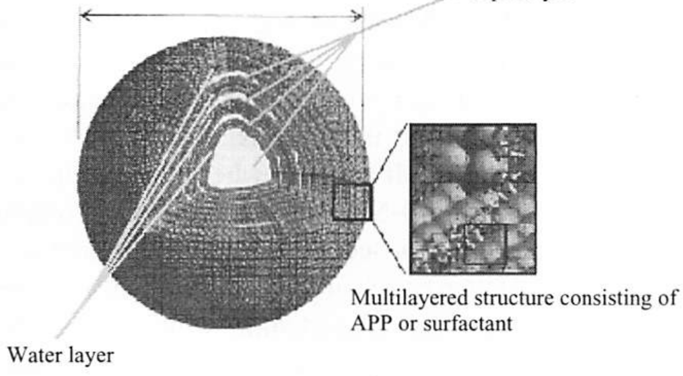

Figure 4 Multilayered structure of APP liquid crystal

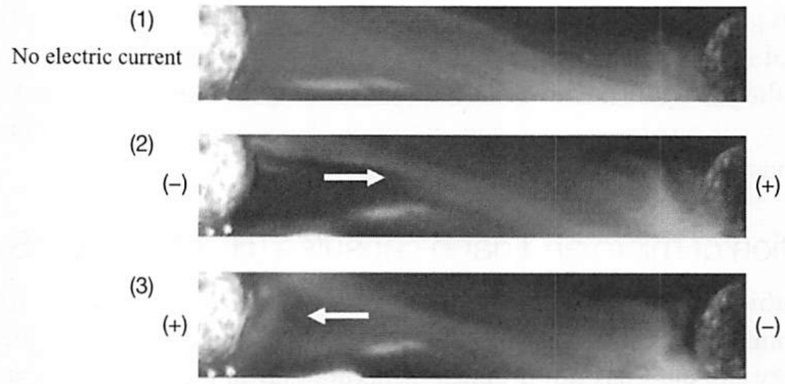

Figure 5 Electrophoresis of APP capsules

Table 2 Kinds and uses of nanomics

Brand name	Main active ingredients	Target active oxygen	Target
Nanomic C	APP	Hydroxy radical	Whitening, acne
Nanomic C + E	APP, Water-soluble vitamine E	Hydroxy radical	Antiaging
		Superoxide	Hair loss, menopause
Nanomic Q	Coenzyme Q_{10}	Superoxide	Dry skin
	Biotin		Atopic dermatitis
Nanomic A	Astaxanthine	Singlet oxygen	Wrinkle
Nanomic F	Fullerene	Various kinds of active oxygen	Antiaging

solutions and improved stability of lipids contained in the capsules. Particularly the APP capsules that contained various kinds of water-soluble and lipid-soluble antioxidants showed especially high improvements in the stability of the APP and the antioxidants. We estimate that the stabilization of both the APP film and antioxidants by the ITO-Nano DDS capsule was attributable to the artificial reproduction of the redox balance system of antioxidants in living cells.

Synergy with fullerene

Fullerene is frequently described as a "nanocapsule" because it has a shape of a "bird cage". Enclosure of hydrogen and other molecules in fullerene has recently been introduced by the media and enhanced the image of being a nanocapsule. However, cosmetic articles containing fullerene on the market are unrelated to nanotechnology. Today, fullerene is added to cosmetics as an antioxidant because it can stably absorb a large quantity of activated oxygen and has received a nickname of being a "radical sponge". In most cosmetics, fullerene is added as an antioxidant like vitamins C and E, and the use has no relationship with nanotechnology. However, we obtained an interesting result recently when we examined the ITO-Nano DDS capsule properties of fullerene. When ITO-Nano DDS capsule that contained an ascorbic acid derivative at a high concentration was exposed to a radical environment, such as UV, radicals of ascorbic acid were produced. However, when fullerene was added to the nanocapsules, the generation of ascorbic acid radicals was reduced significantly. Our experiment showed that fullerene controlled the generation of ascorbic acid radicals by UV under the presence of ascorbic acid at high concentrations. Fullerene was also found to inhibit the decomposition of carotenoids, such as β carotene and astaxanthine, under a hyperoxidation condition of lipids. We also found that fullerene inhibited the generation of superoxide radicals in the skin by infrared laser irradiation at 1440 nm significantly. Our experiments suggested that fullerene inhibits oxidization of both water-soluble and lipid-soluble antioxidants, the property of which possibly acts synergistically with amphipathic vitamins such as APP.

Conclusions

Many derivatives of ascorbic acid are used today in various fields, of which APP is of special interest because it also has properties of vitamin C unlike the other derivatives. The amphipathic properties of APP are likely promising tools for developing anti-aging systems with an effective redox balance, in which water-soluble and lipid-soluble redox molecules resonate with each other, and for applications in diverse industrial fields, including cosmetics and medical supplies.

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