BIOLICAL ACTIVITIES OF A STABLE ASCORBIC ACID DERIVATIVE, 2-0-α-D-GLUCOPYRANOXYL-L-ASCORBIC ACID (AA-2G) IN COSMETICS

Osamu Moro, Ph.D.
Shiseido Research Center, 1050 Nippa-cho, Kohoku-ku, Yokohama-shi, Kanagawa, JAPAN 223-8553

Received: May 29, 1999

Key words: Ascorbic acid, Vitamin C, 2-0-α-D-glucopyranosyl-L-ascorbic acid, Melanogenesis, Collagen, Antioxidation.

Summary

Ascorbic acid or vitamin C has become a popular ingredient in cosmetics for several reasons. Its image is one of a healthy and safe product and it has important biological functions in the skin. However, ascorbic acid is less stable than other vitamins used in cosmetics as it rapidly decomposes and becomes inactive. Efforts have been made to generate analogs that are more stable and maintain the positive properties of ascorbic acid. The ascorbic acid derivative, ascorbic acid 2-0-α-D-glucopyranosyl-L-ascorbic acid (AA-2G) is stable in a neutral solution and is hydrolyzed into ascorbic acid and glucose by an enzyme present in skin. AA-2G exhibits prolonged effects on collagen synthesis, inhibition of melanogenesis and functions as an antioxidant. All of these activities are inhibited by the presence of the α-glucosidase inhibitor castanospermine indicating that ascorbic acid released by hydrolysis of AA-2G is the active principal. Taken together, these results suggest that AA-2G will be an effective ascorbic acid derivative in cosmetics.

Riassunto

L’acido ascorbico o vitamina C è diventato un ingrediente popolare nei prodotti cosmetici per molte ragioni. E’ uno dei prodotti più utili per la salute, svolge importanti funzioni biologiche per la pelle ed è sicuro nell’uso. Comunque l’acido ascorbico è meno stabile di altre vitamine d’uso cosmetico, perché si decompono rapidamente diventando inattivo. Molti studi sono stati condotti per produrre derivati più stabili che mantenessero le proprietà dell’acido ascorbico. Il 2-O-α-D-glucopiranosoil-L-ascorbato (AA-2G) è stabile in soluzioni neutre ed è idrolizzato ad acido ascorbico e glucosio da un enzima presente a livello cutaneo. AA-2G svolge un positivo effetto sulla sintesi del collagene, inibisce la melanogenesi e funge da antiossidante. Tutte queste attività sono regolate da un enzima specifico che inibisce l’attività dell’acido ascorbico, a dimostrazione che è tale acido il composto attivo. Tutto ciò sta a indicare che l’AA-2G è un derivato della vitamina C efficace a livello cutaneo.
INTRODUCTION

Ascorbic acid has a variety of physiological functions in biochemical reactions including synthesis of collagen and polysaccharides, metabolism of tryptophan, phenylalanine and tyrosine, absorption of iron, steroid hydroxylation, and antioxidation (1). However, the human being is unable to synthesize ascorbic acid due to the lack of L-gulonolactone oxidase which is required for the conversion of L-gulonolactone to L-ascorbic acid. Therefore, we need to take in approximately 100 mg/day in order to maintain a normal ascorbic acid level (2). Although ascorbic acid is usually administered orally as a nutritional supplement, it has been also formulated in a number of cosmetic products since some of the physiological functions of ascorbic acid make its use in cosmetics attractive. These functions include inhibition of melanin synthesis, promotion of collagen synthesis and antioxidation. Moreover, ascorbic acid is safe and has been shown to be absorbed through the skin despite its high hydrophilicity (3).

For cosmetics formulators, a major drawback of ascorbic acid is its instability in aqueous formulations. Ascorbic acid is easily oxidized by oxygen in aqueous solutions, resulting in rapid degradation and deterioration in color of the products. A variety of pharmaceutical preparations including W/O type emulsion, liposome and cyclodextrin have been used to improve the stability of ascorbic acid in formulations. Despite these efforts, a definitive solution to the problem of oxidation of ascorbic acid has not been found. Several ascorbic acid derivatives such as ascorbic acid 6-palmitate, ascorbic acid 2,6-dipalmitate, ascorbic acid 6-stearate, 2,3,5,6-O-tetra-2-hexyldecanoyl L-ascorbic acid (VC-IP) have been developed. These modifications have improved penetration into the skin, but they do not have improved stability in an aqueous environment than the parent compound. In order to improve stability, the hydroxy group at positions 2 and/or 3 must be protected. Because the physiological function of ascorbic acid stems from this enediol system, ([(-C(=O)OH)=C(=O)-]), these modifications make the ascorbic acid derivatives physiologically inactive. In order to achieve both stability in the formulations and physiological efficacy, the hydroxy groups at position 2 and/or 3 need to be protected but, upon application to the skin, these compounds should be hydrolyzed by enzymes in skin to generate active ascorbic acid.

Yamamoto et al. found that the homogenates of the small intestine and kidney of rats and guinea-pigs had a transglucosylase activity that forms a new type of glucosylated ascorbic acid (4,5). This novel compound was stable in a neutral solution and did not possess reducing activity. These properties are consistent with those of ascorbic acid 2-O-phosphate (AA-2P) (6,7) and ascorbic acid 2-O-sulfate (AA-2S) (8). The reducing activity was restored by mild acid hydrolysis or treatment with rat intestinal a-glucosidase. The structure of this novel compound was found to be 2-O-α-D-glucopyranosyl L-ascorbic acid (9). A large scale preparation of AA-2G has been made possible by using cyclo-maltodextrin glucanotransferase (CGTase) prepared from bacillus stearothermophilus which catalyze the transglycosylation from α-cyclodextrin to L-ascorbic acid (10). In this paper, the biological activities of 2-O-α-D-glucopyranosyl-L-ascorbic acid (AA-2G) and its application in cosmetics are discussed.

SUSTAINED RELEASE OF ASCORBIC ACID FROM AA-2G

In order to exhibit physiological activities, ascorbic acid 2-O derivatives should be hydrolyzed in the skin. Both α-glucosidase and alkaline phosphatase activities have been detec-
As:orbic acid 2·0·glucose (AA·2G)

U: Molecular Structure

Enzyme to release AA

Enzyme activity in the skin

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<td>Fig. 1 Ascorbic acid 2-O-derivatives.</td>
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<th>Ascorbic acid 2-O-glucoside (AA·2G)</th>
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Sustained ascorbic acid release from AA-2G may be due to the moderate α-glucosidase activity in skin. Another possible explanation is feedback control of α-glucosidase activity. AA-2G is hydrolyzed into ascorbic acid and glucose by α-glucosidase exist in cell membranes. The uptake of ascorbic acid by its transporter is inhibited by released glucose. As a result, the extracellular ascorbic acid concentration increases and α-glucosidase activity is suppressed by feedback control. However until now, there was no evidence for the inhibition of ascorbic acid uptake by glucose, while dehydroascorbic acid uptake has been reported to be inhibited by glucose at the site of dehydroascorbic acid transporters (18,19). Na⁺-dependent L-ascorbic acid transporters have recently been cloned (20).
These results will facilitate the understanding of ascorbic acid transport in various organs including skin, at a molecular level.

**COLLAGEN SYNTHESIS**

Collagen and elastin are two major extracellular matrix molecules in the skin. Collagen provides a scaffold for skin structure. In scurvy, a condition that results from vitamin C deficiency and thus decreased collagen synthesis, the skin becomes dry and rough. A reduction in collagen synthesis and the relative change in amounts of collagen isoforms type I and III also occurs in photoaging (21,22). Both UVA and UVB irradiation was shown to increase MMP-1 (intestinal collagenase) mRNA steady-state levels (23,24). Ascorbic acid act as a cofactor for the hydroxylation of proline to hydroxyproline which acts to stabilize the collagen triple helix. Accumulated evidence indicates that ascorbic acid is not only involved in the hydroxylation of proline, but is also involved in collagen gene transcription and steady-state levels of collagen mRNA (25-28) Tajima and Pinnell demonstrated that 72 h treatment of 100 mM ascorbic acid caused a 3-4 fold enhancement in the transcription of types I and III collagen genes in skin fibroblast culture, as determined using nuclear run-on experiments (28). AA-2G dose dependently stimulates collagen synthesis in human skin fibroblasts (14). Sustained stimulation of collagen synthesis has also been observed for AA-2G. A one-time addition of 0.25 mM AA-2G caused a definite stimulatory effect over 5 days whereas ascorbic acid lost activity after a 5 day cultivation period (Fig. 3) (14). Inhibition of collagen synthesis after 5 days was not observed by the frequent addition of ascorbic acid indicating that the long-lasting effect of AA-2G is
due to its slow release of ascorbic acid (17). The effect of AA-2G on collagen synthesis was inhibited in the presence of the a-glucosidase inhibitor castanospermine indicating that ascorbic acid released by hydrolysis of AA-2G was responsible for collagen synthesis (17).

**MELANOMEGENESIS**

Tyrosinase inhibitors such as arbutin, 4-butyresorcinol, kojic acid and ellagic acid are widely used in cosmetics as whitening agents in Japan. Arbutin and 4-butyresorcinol are competitive inhibitors while kojic acid and ellagic acid are non competitive inhibitors that chelate the Cu ion required for tyrosinase activity. Ascorbic acid is not a tyrosinase inhibitor but exhibits a whitening effect due to its strong reducing properties. In the melanogenic pathway, tyrosinase catalyses the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylanine (DOPA) and the oxidation of DOPA to DOPA quinone. Ascorbic acid has been shown to inhibit melanin formation by reducing DOPA quinone back to DOPA (Fig. 4). It is reported that the addition of 0.1 mM ascorbic acid slowed down the DOPA chrome production and 0.5 mM ascorbic acid completely inhibited DOPA chrome production (29). An inhibitory effect on melanin synthesis in B16 melanoma was observed over a period of 15-20 h following the addition of 2 mM AA, AA-G and AA-2P to the culture medium. After 15-20 h, a rapid increase in melanin synthesis was observed in cells treated with AA and AA-2P but not AA-2G. AA-2G exhibited this inhibitory effect for more than 30 h (Fig. 5) (17). Akiyama et al. examined the anti-pigmentation effect of AA-2G in vivo. They reported a significant prevention of both erythema and pigmentation by a 2% AA-2G containing cream after
exposure to a mixed UVA and UVB radiation to the inside of human upper arm (30).

**ANTIOXIDATION**

The concept of antioxidation is important in cosmetics as the skin is always in contact with harmful external stimuli including oxidizing substances and sunlight. The structural and functional alteration of skin compounds caused by UVB induced reactive oxygen species is thought to contribute to photoaging. Cutaneous antioxidans include α-tocopherol (vitamin E), ascorbic acid and reduced glutathione. Antioxidant enzymes in skin that counteract reactive oxygen species include superoxide dimutase, catalase and glutathione peroxidase. The hydroxyl radical is thought to be most potent oxidant and reacts with a number of compounds including enzymes, carbohydrates, lipids and DNA. α-tocopherol quenches hydroxy radicals by donating a hydrogen to them. Ascorbic acid reduces α-tocopherol back to an active form by donating a hydrogen. In addition to regenerating α-tocopherol from the tocoperoxy radical form, ascorbic acid itself scavenges reactive oxygen species. Topically applied ascorbic acid and α-tocopherol seem to be effective only when applied before UV exposure indicating that a sufficient concentration should be present at the site of action during oxidative stress to prevent UV induced skin damage (31,32). Pretreatment of keratinocytes by 0.2 mM AA-2G prevented a decrease in viable cell number by UVB radiation (17,33). AA-2G reduced efficiently the cytotoxicity of H₂O₂ (17). Lipid peroxidation induced cytotoxicity was not prevented by AA-2G itself. However, AA-2G significantly increased the protective effect of α-tocopherol. These results are consistent with the previously mentioned functions of ascorbic acid in antioxidation in both scavenging reactive oxygen species and regenerating oxidized tocopherol and suggest that AA-2G can reduce actinic injury. Likewise, it has also been reported that application of a cream containing AA-2G decreased the number of sunburn cells caused by UVB radiation (17).
CONCLUDING REMARKS

Two major problems associated with the formulations containing ascorbic acid have been instability and short-lasting effects in the area to which the cosmetic was applied. It is now possible to circumvent these problems by using AA-2G. Recently, Yamamoto et al. developed next generation of AA-2G in which an acyl group is added to the hydroxy group at position 6 (6-O-acyl-2-O-α-D-glucopyranosyl-L-ascorbic acid). This modification facilitates absorption through the skin and a more enhanced effect of ascorbic acid is expected (34). Human skin is subject to harmful external stimuli. These stimuli include oxidizing substances and sunlight. The effects of these harmful agents leads to ageing of the skin, manifest as wrinkles and pigmented spots. As we move into the 21st century, the number of elderly people will increase. These people will desire cosmetics that slow, halt or reverse the ageing process. Derivatives of ascorbic acid will surely enjoy continued growth in the field of cosmetics.

Fig.5 Reducing action of AA-2G on melanin synthesis. (Kumano et. al. J. Nutr. Sci. Vitaminol. 1998)
REFERENCES


Corresponding Author:
Osamu Moro, Ph.D.
Shiseido Research Center
1050 Nippa-cho, Kohoku-ku
Yokohama-shi, Kanagawa
JAPAN 223-8553
Tel: + 81 45 545 3407
Fax: + 81 45 545 3339
e-mail: osamu.moro@to.shiseido.co.jp