

Physicochemical, Biochemical and Antioxidant Properties of Ascorbic Acid

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ABSTRACT

Ascorbic acid (Vitamin C) is an important micronutrient that plays as a redox cofactor and catalyst in biological system. It is particularly useful in the cure and prevention of scurvy. It was originally isolated from lemon, cabbage and pepper. Ascorbic acid is essential for collagen formation and metabolism of phenyl alanine, tyrosine and hydroxyphenyl alanine (dopa). The minimum daily requirement of vitamin C in adult is 40-60 mg. In this review chemical structure, physicochemical characteristics, chemical stability, biochemical functions and antioxidant activity of ascorbic acid have been mentioned.

Key words: Ascorbic acid, redox, collagen, scurvy.

INTRODUCTION

Vitamin C has long been recognized as an important nutrient in several food products. Ascorbic acid plays as a redox cofactor and catalyst in biological system. Vitamin C (ascorbic acid) has an ability to cure and prevent scurvy. The disease scurvy is due to a deficiency of ascorbic acid in diet, and has considerable significant history.¹⁻⁶ The antiscorbutic activity factor isolated from a crude fraction of lemon and showed that the activity was destroyed by oxidation and protected by reduction. A reducing agent in pure form is isolated from cabbage.⁷ The compound identified as the active antiscorbutic factor in lemon juice.⁸ The chemical name ascorbic acid was assigned to the substance to designate its function in preventing scurvy.⁹ The most important function of ascorbic acid is its essential presence of the formation collagen, ground substances, osteoid, dentine and intercellular cement substances. It is vital for collagen formation at several points e.g., preservative and maturation of fibroblast and elaboration of hydroxyproline and hydroxylysine.^{10,11} Ascorbic acid is necessary for the normal metabolism of phenyl alanine, tyrosine and hydroxy phenyl alanine (dopa). The minimum amount of ascorbic acid necessary to prevent scurvy for one year and yet maintain good healing properties of tissues appears to be slightly less than 10 mg daily.¹² Ascorbic acid facilitates the absorption of iron by keeping the iron

in the reduced form. A few microcytic anemias respond to ascorbic acid treatment, which may be in part due to improved absorption of iron.

Ascorbic acid is widely distributed in the active tissue of higher plants. Almost all fresh fruits and tubers contain significant amount of the vitamin. Orange and lemon juices are outstanding source of ascorbic acid and contain approx 0.5 mg/ml. It is also manufactured synthetically and is extensively used in the form of tablets and as an ingredient of multivitamin preparation.

Plants and most animals synthesize their own vitamin C but human lack this ability due to the deficiency in an enzyme, L- gulono-gamma- lactone oxidase that catalyzes the terminal step in ascorbic acid.¹³ Therefore, humans obtain this vitamin from diet and /or vitamin supplement to not only avoid the development of scurvy but also for overall well being.¹⁴⁻¹⁶ The minimal daily requirement of ascorbic acid in adults is 40-60 mg.¹⁷⁻²⁰

PHYSICOCHEMICAL CHARACTERISTICS

The important physicochemical characteristic of ascorbic acid (Table1) involved in its identification, determination and degradation are described by many authors.²¹⁻²⁵ The redox property is the basis for its physiological activity. In addition the proton on oxygen-3 is acidic (pKa1= 4.17) which contributes to an acidic

nature of ascorbic acid.

CHEMISTRY

Nomenclature and structure

The IUPAC-IUB Commission on Biochemical Nomenclature changed vitamin C (2-oxo-L-theohexono-4-lactone-2,3-enediol) to ascorbic acid or L-ascorbic acid in 1965.²⁵

The chemical structure of ascorbic acid (1) is

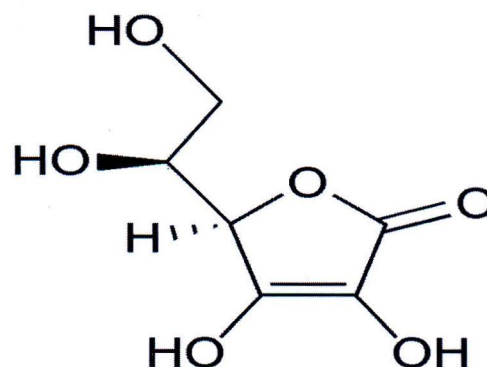


Table-1 Physicochemical characteristics of Ascorbic Acid

Form and colour	Solubility	Specific optical rotation	Partition coefficient	Dissociation constant	UV and visible spectrum	Infra red spectrum	Mass spectrum
Colorless crystals or a white or almost white crystalline powder which discolors on exposure to air and moisture. Odorless or almost colorless. ²⁶ Colorless crystals or a white or almost white crystalline powder which discolors on exposure to air and moisture. Odorless or almost colorless. ²⁶	Soluble in 1.3 of water, very slightly soluble in ethanol (96%), practically insoluble in chloroform and in ether. ²⁶	In a 10% w/v solution +20.50 to +21.50. ²³	Log P (octanol/water), 1.8. ²³	pKa 4.2, 11.6 (25°C). ²³	Absorption Maximum [A(1%, 1 cm)] pH 2.0 245nm[695] pH 6.0 265nm [940] ²³	Principal ions at m/z 29, 41, 39, 42, 69, 116, 167, 168. ²³	Principal peaks (Nujol mull) 1026 (C-OH str), 1111(C-O-C str), 1312 (-C-O-str), 1653 (C=O str), 990 cm ⁻¹ (C=C str) ²³

Chemical Stability

Ascorbic acid is sensitive to air and light and is kept in a closed container, protected from light.²⁶ The most important chemical property of ascorbic acid is the reversible oxidation to semidehydro-L-ascorbic acid and oxidation further to dehydro-L-ascorbic acid. This property is basis for its physiological activity. Degradation reaction of L-ascorbic acid in aqueous solution depends on a number of factors such as pH,

temperature, presence of oxygen or metals. In general, ascorbic acid is not stable in aqueous medium at room temperature due to oxidation to dehydroascorbic acid. The stability of ascorbic acid and dehydroascorbic acid can be improved by lowering the pH below 2.²⁷ Above this pH alkali-catalyzed degradation by cleavage at C-1 OR C-2 results in a number of compounds mainly mono-, di-, and tri carboxylic acid.^{21,28,29} The oxidative degradation of ascorbic acid and

dehydroascorbic acid in parenteral nutrition mixture is catalyzed by trace elements particularly, copper.³⁰⁻³⁴ Stabilized ascorbic acid preparations have been reported in hydroalcoholic vehicle³⁵ and aqua culture feed³⁶. It is noted that, in addition to redox and acid-base properties ascorbic acid can exist as a free radical.^{37-39, 28} The ascorbate radical is an important intermediate in the reaction involving oxidants and ascorbic acid antioxidant activity. Rate constant for the generation of ascorbate radical are in range of 10^4 - 10^8 s⁻¹. When ascorbate radical are generated by oxyanions the rate constants are of the order of 10^4 - 10^7 s⁻¹, when generated by halide radicals, 10^6 - 10^8 s⁻¹, and when generated by α -tocopherol and flavanoids radicals, 10^6 - 10^8 s⁻¹.^{29,38} The ascorbate radicals decay usually by disproportionate. However, a change in ionic strength and pH can influence the rate of dismutation of ascorbic acid. Certain oxyanions such as phosphate accelerate dismutation.

Ascorbic acid is incompatible with sodium salicylate, sodium nitrite, and theobromine and methanamine.⁴⁰ The outstanding feature of the structure of ascorbic acid is presence of α -ketodiol system involving carbons 1, 2 and 3 as shown in Fig-1 This portion of the molecule is responsible for the characteristic ultraviolet absorption of the compound. Ascorbic acid is an unsaturated lactone (cyclic ester) and in aqueous solution it is easily oxidized to give dehydroascorbic acid which has full vitamin activity. The dehydroascorbic acid can undergo further hydrolysis to give an irreversible degradation products diketogulonic acid and oxalic acid. Maximum stability of ascorbic acid occurs near pH-3 and pH-6.^{21,41,42}

The anaerobic degradation of ascorbic acid has been studied by.⁴³ Under these conditions the molecule dehydrated and hydrolysed in aqueous solution to give furfural and carbon dioxide. The rate of degradation is maximum at pH 4.1, corresponding to the pKa of ascorbic acid. This has been suggested due to the formation of a salt-acid complex in solution. The reaction is dependent on buffer concentration but has relatively small effect of ionic strength.

The juice matrix composition of ascorbic acid and

fortification effect on the phytochemical and antioxidant. The presence of non-anthocyanin poly phenolics exerted a protective effect against ascorbic acid oxidation and antioxidant stability during storage.^{44,45}

In the moisture induced solid phase degradation of ascorbic acid. The degradation give rise to the discoloration of the samples. The most surely degraded samples are almost black in appearance. The samples were analyzed in the presence of carbonyl compounds which are responsible for discoloration and evolution of carbon dioxide. In the reaction one mole of carbon dioxide was evolved per mole of ascorbic acid⁴⁶ and determination of stability of ascorbic acid and dehydroascorbic acid in blood plasma and serum was studied by.^{47,48}

BIOCHEMICAL FUNCTIONS

An important biological function of ascorbic acid is likely to be its reaction with destructive free radical particularly those derived from oxygen. Ascorbate ion can also enhance oxidative damage to macromolecules, in vitro, especially in the presence of Fe²⁺ or Cu²⁺ ions.

Ascorbic acid plays an essential role in the activities of several enzymes. It is vital for the growth and maintenance of healthy bones, teeth, gums, ligaments and blood vessels. It is important for the manufacture of neurotransmitters and adrenal hormones. Ascorbic acid is required for the utilization of folic acid and the absorption of iron. It is also necessary for normal immune responses to infection and the wound healing.⁴⁹ Ascorbic acid deprivation and scurvy include a range of signs and symptoms that involve defects in specific enzymatic processes.²⁵ The administration of ascorbic acid improves most of the signs of chemically induced glutathione (L- α -glutamyl-L-cysteine-glycine, GSH) deficiency.⁵⁰ The effect is very pronounced in new born rats which do not efficiently synthesize ascorbic acid in contrast to adult rats and guinea pigs. When L- buthionine-(SR)-sulphoxime is administered. In addition to the loss in GSH, there is a marked increase in dehydroascorbic acid. This has led to the hypothesis that GSH is very important to

dehydroascorbic acid reduction, and as a sequence, to ascorbic acid recycling.⁵¹

Ascorbic acid also possesses pro-oxidant properties and may cause apoptosis lymphoid and myeloid cells. It has been shown that dehydroascorbic acid also stimulates the antioxidant defenses in some cells by preferentially importing dehydroascorbate over ascorbate.⁵²⁻⁵⁵

ANTIOXIDANT ACTIVITY

Antioxidants are a group of substances, which, at low concentrations, significantly inhibit or delay oxidative processes, while often being oxidized themselves. The generally accepted hypothesis is that in any biological system, an important balance must be maintained between the formation of reactive oxygen species and reactive nitrogen species. The reactive species such as superoxide (O₂), hydrogen peroxide (H₂O₂), hydroxyl radical (HO·), nitrogen oxide (NO·), peroxy nitrite (ONOO·) and hypochlorous acid (HOCl) are all products of normal metabolic pathways of the human organs but, under certain conditions, when in excess, they can exert harmful compounds.

Ascorbic acid is known to readily scavenge reactive oxygen and nitrogen species, such as superoxide and hydroperoxyl radicals, aqueous peroxy radicals, singlet oxygen, ozone, peroxy nitrite, nitrogen dioxide, nitroxide radicals and hypochlorous acid. Excess of such products has been associated with lipids,⁵⁶⁻⁵⁸ DNA⁵⁹⁻⁶¹ and protein oxidation.⁶²⁻⁶⁶

Mechanism

Naturally occurring antioxidants, of high or low molecular weight, can differ in their composition, their physical and chemical properties and their mechanism and site of action. High molecular weight protein such as albumin, ceruloplasmin, transferrin and hepatoglobin, which are all present in plasma, bind to redox-active metals and limit the production of metal catalyzed free radicals. Albumin and ceruloplasmin can bind copper ions and transferrin binds with iron. Hepatoglobin binds heme containing protein and can thus clear them from circulation. Low molecular weight antioxidants are sub divided

into lipid-soluble antioxidants (α -tocopherol, carotenoids, quinones and some polyphenols) and water soluble antioxidants (ascorbic acid, uric acid and some polyphenols). They delay or inhibit cellular damage mainly through their free radical-scavenging property. The major water soluble low molecular weight antioxidants in human plasma are ascorbic acid and uric acid. Vitamin C act as strong antioxidant in the plasma and presents a synergistic effect with other antioxidants (co-antioxidant).

Two principle mechanism of action have been proposed for antioxidants. The first is chain breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the system (e.g., lipid radical).

The second mechanism involves removal of reactive oxygen and nitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalysts.⁶⁷

Electron Donation

The electron donor character of ascorbate may be responsible for many of the known biological functions. In spite of the availability of ascorbic acid to influence the production of hydroxyl and alkoxy radicals, it remains uncertain whether this is the principle effect or mechanism that occurs in vivo. There is good evidence that ascorbic acid protects lipid in biological fluid as an antioxidant.²⁵ A detailed account of the function of ascorbate as an antioxidant has been reported and its reaction with reactive nitrogen species and singlet oxygen.⁶⁸⁻⁶⁹ Ascorbic acid (E° -0.115 V, pH 5.2,²⁴ has been used as an antioxidant for the stabilization of drugs with higher oxidation potential. These drugs include morphine,⁷⁰ vitamin A,⁷¹ rifampin,⁷² cholecalciferol,^{73,74} promethazine⁷⁵ and sulphacetamide and sulphanilamide.⁷⁶

Co-Antioxidant

Ascorbic acid alone has little effect in preventing lard oil from oxidation, it has been reported that the combination of ascorbic acid with α -tocopherol give rise to a strong synergistic antioxidative effect.⁷⁷ These authors conclude that role of ascorbic acid was to

preserve α -tocopherol from consumption. This behavior of ascorbic acid is termed as co-antioxidant effect. Since, then many other compounds have been found to produce a similar co-antioxidant effect with α -tocopherol.

CONCLUSION

Ascorbic acid is an important nutrient for food material and important for redox processes in the biological system. It is essential for cure and prevention of scurvy as well as for collagen formation and skin health care. It is widely distributed in the active tissues of higher plants, fruits and vegetables. Ascorbic acid is sensitive to air and light and undergoes oxidation to dehydroascorbic acid and further products. Ascorbic acid possessed antioxidant property and readily scavenge reactive oxygen and nitrogen species including superoxide, hydroperoxyl radicals, aqueous peroxy radicals, singlet oxygen, ozone, peroxy nitrite which are associated with the damage of lipids, DNA and proteins. The combination of ascorbic acid with α -tocopherol give rise to strong synergistic antioxidant effect.

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