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Structure of Ascorbic Acid and Its Biological Function

VI. Its Importance for the Na^+/K^+-Transport

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Abstract. Ascorbic acid/isoascorbic acid are present as radicals at physiological pH with the unpaired electron located in the C(4) region. Since a distinction can be made between both types of radicals, the electron spin resonance technique can be used for discrimination between the epimers of vitamin C. The radical has a cyclic side-chain structure which is formed by the hydrogen bond $C(3)-O^- \ldots HO-C(6)$ (≈ 2.7 kJ) and which engulfs Na^+ or K^+ in the case of the ascorbyl or the isoascorbyl radical, respectively. The radicals Na-ASC and K-Iso-ASC are electroneutral. Red. glutathione affects both types of radicals by restoring the original electronic configuration at C(4) without changing the electroneutral bicyclic structure. In this way, the mobile carriers Na-ASC and K-Iso-ASC can transport Na^+ and K^+ across membranes. Its highest efficiency is around 37° C and pH $\approx$ 7, that is, at physiological values. The biological importance of the side chain of vitamin C is outlined and a possible transport mechanism proposed.

Key words: Ascorbic acid – Na^+/K^+-transport – ESR

Introduction

Ascorbic acid is known as an essential substance in the biochemistry of living cells (Szent-Györgyi 1979). It serves as a reductant or cofactor for certain enzymatic reactions and is a component of a general redox buffer system. The presence of the ascorbyl radical in certain types of tumors (Lohmann et al. 1979, 1982) and its involvement in lipid peroxidation (Haase and Dunkley 1969) also suggest a major biochemical role of ascorbic acid. The molecular details of oxidation and reduction and the accompanying structural changes, however, have not yet been completely elucidated (Lewin 1976).

Recently, Lohmann et al. (1983a, b, c), Lohmann and Holz (1983) and Lohmann and Winzenburg (1983) have tried to obtain more information about the structure and, thus, the function of ascorbic acid. This report will combine

these findings to outline a detailed structure with its implications for the biological function of ascorbic acid.

Material and Methods

The materials and methods used have been described elsewhere (Lohmann et al. 1983 a, b, c; Lohmann and Holz 1983; Lohmann and Winzenburg 1983).

Results and Discussion

In a solid matrix, the ascorbyl radical usually exhibits a doublet (Laroff et al. 1972), while in aqueous solution an additional hyperfine structure can be observed (Lohmann and Holz 1983; Sapper et al. 1982).

The doublet is the result of the interaction between an unpaired electron, produced by the cleavage of an H-atom belonging to a tightly bound water molecule in the C(4) region, and either one of the protons attached to C(4) or C(5). This cleavage can be produced, for example, by lyophilization.

In the electron spin resonance (ESR) spectra of many lyophilized tissues the shape of the ascorbyl radical shows a slight asymmetry which is influenced by the concentration ratio of Na^+/K^+ (Lohmann and Holz 1983). While the plasma ESR spectrum reflects solely the Na-ascorbyl radical, the other spectra are affected by a certain Na^+/K^+ ratio.

To get more information on the involvement of Na^+ and K^+, the ascorbyl radical has been investigated in aqeous solution at room temperature. These investigations (Lohmann and Holz 1983) revealed that with increasing pH, the ascorbyl radical is formed. Its concentration exhibits an optimum at around pH 7 and decreases rapidly at larger values.

In addition to the cleavage of the H atom mentioned above for a solid matrix, the hydroxyl ions lead to the removal of the proton of the OH group attached to C(3). This will affect the electron densities at C(3) and C(4), resulting in a considerable upfield shift of H-4 and downfield shift of C(3) (Lohmann et al. 1983a).

At pH $\approx$ 7, ascorbic acid (ASC; with a free side chain) has been converted to the radical configuration (Na-ASC) (Lohmann and Holz 1983), which has a cyclic side-chain structure (Sapper et al. 1982). The ring closure will occur via a hydrogen bond formation between $C(3)-O^-$ and C(6)–OH. This result is also supported by the IR findings, according to which the two O–H stretching bands originating from the OH groups attached to C(3) and C(6) are missing at pH 7, that is, in the radical state (Lohmann et al. 1983b).

The change in electron densities at C(3) and C(4) is also expressed by a shift of the C = O and C = C stretching bands towards smaller wave numbers (Lohmann et al. 1983b). This result is confirmed by the UV absorption spectra, which exhibit a red shift (245 $\rightarrow$ 264 nm) of the $\pi-\pi^*$ excitation of the C = C double bond with the formation of the radical (Lohmann et al. 1983b).

It should be pointed out that the wavelength shift occurs within a rather small concentration range of ASC ($50 \cdot 10^{-5} \rightarrow 4 \cdot 10^{-5}$ M: 245 → 264 nm) concomitantly with a change in pH (3 → 6.8). At constant ASC concentration, e.g., 1 mM, addition of NaOH also results in a pH increase with a concomitant increase in λ_{max} (at pH 7, $\lambda_{max} \approx 263$ nm).

Similar results were obtained with K^+ which seems to be specific for isoascorbic acid (Iso-ASC), while Na^+ is specific for ASC. This is also demonstrated by the finding that addition of NaOH to Iso-ASC leads to a precipitation above pH ≈ 4.

Furthermore, there is a difference in the splitting of the doublet and its hf structure between ASC and Iso-ASC (Lohmann and Holz 1983). There are two different triplets and this thus allows easy and fast discrimination between the two epimers of vitamin C. Additional splittings in the ESR spectra, which might be due to the hf interaction with the nuclear spins of Na^+ and K^+, can be observed. Because of poor resolution, an assignment could not be given.

The Na-ASC and K-Iso-ASC radicals formed are electroneutral (Lohmann et al. 1983c) (probably due to a 1 : 1 ASC: Na^+ or Iso-ASC: K^+ ratio). This and the cyclic side-chain structure should enable the Na-ASC and K-Iso-ASC radicals to permeate membranes even at room temperature. This could be confirmed by experiments on DPPC (dipalmitoylphosphatidylcholine) vesicles, in which the radicals reduced the spin label I (1,14) located at the apolar end of the CH_2 chain (Lohmann et al. 1983d). The mechanism involved is shown in Fig. 1. The hydrophilic free side-chain prevents the permeation of ASC or Iso-ASC across membranes. When, however, the bicyclic structure is formed (radical) and the total charge is zero, permeation is achieved.

Since the ascorbyl radical leads to lipid peroxidation and, thus, destruction of the membrane (Haase and Dunkley 1969), restoration of the original electronic configuration at C(4) without changing the electroneutral bicyclic structure is required. The presence of red. glutathione (GSH) in the membranes will reduce the radical state and yet allow the mobile carriers Na-ASC and K-Iso-ASC to transport Na^+ or K^+ across the membrane. There is no difference in permeation rate of Na-ASC treated with or without GSH (Lohmann et al. 1983d).

Such a transport has its highest efficacy at around 37° C, the physiological temperature (Lohmann et al. 1983d; Lohmann and Holz 1983). Higher temperatures (> 50° C) result in a rapid decrease in spin concentration caused by breaking up the hydrogen bond forming the furanoid ring.

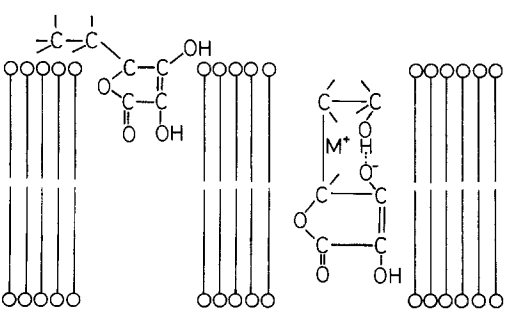

Fig. 1. Model proposed for the transport of ascorbic acid and alkaline ions (M^+: Na^+, K^+) across membranes. If side chain is not closed (left side), transport is inhibited

These results also allow the strength of the hydrogen bond to be estimated. Since this bond will break up at about 50° C, its energy is about 2.7 kJ. Thus, the energy requirement for the release of Na^+ or K^+ after transport across the membrane is minimal, that is, the efficacy of the biological system is optimal.

This small energy requirement might be the reason for an additional observation: a Na-ASC sample measured repeatedly in the ESR spectrometer, loses its spin concentration rapidly. Oxygen influence can be neglected, since a new sample taken from the stock solution, exhibits, at the first determination, the original spin concentration followed by a rapid decay during repeated measurements. From this it might be concluded that additional microwave energy is sufficient enough to breake up the hydrogen bond by heating the sample.

From the results obtained one might conclude that Na^+ and K^+ are transported by the mobile carriers ASC and Iso-ASC, respectively, across the artificial membrane, the DPPC vesicles. The structure proposed for these carriers is shown in Fig. 2. This agrees well with the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra (Lohman et al. 1983a), according to which Iso-ASC exhibits a stronger H-4/H-5 interaction than ASC (Karplus rule). The remaining coupling constants, e.g., $J_{5,6}$ or $J_{5,6*}$, can be explained by the magnetic non-equivalency of the CH_2-6 protons. The "cage" formed by the side chain will be closed by the easily polarizable hydrogen bond $C(3)-O^- \ldots HO-C(6)$; it will engulf either Na^+ (in the case of ASC) or K^+ (in the case of Iso-ASC). The loading of such a carrier can occur only at around pH 7. At this pH, ASC and Iso-ASC are present, however, in their radical configuration. Since such a structure will result in lipid peroxidation, it will be converted to a non-radical structure by GSH in natural membranes in order to avoid this membrane-damaging peroxidation process. The electroneutral cyclic side-chain structure, engulfing Na^+ or K^+, is still maintained; it is a prerequisite for permeating the membrane.

The model proposed can explain the Na^+/K^+ transport across artificial membranes. In natural membrane systems, however, there is active Na^+/K^+ transport. The transport of the Na^+ or K^+ loaded carriers depends on Na/K-ATPase, ATP, GSH, for example. The influence of these substances on the transport of Na-ASC and K-Iso-ASC across the erythrocyte membrane is under investigation at present.

In cell systems, the release of Na^+ or K^+ might be triggered by certain enzyme systems which will break up the hydrogen bond in the furanoid ring.

Under physiological conditions, the stability of the equilibrium between ASC and Iso-ASC and their corresponding semi- and fully oxidized states seems to be maintained by certain enzyme systems. It is suggested that a balance of this complex redox mechanism is required for maintaining optimal physiological conditions and that any unbalance resulting, e.g., in a change in intracellular Na^+ and K^+ (and Ca^{2+}) concentrations, might be a consequence of or might even act as a promoter for a great variety of diseases. A few preliminary investigations have shown drastic changes in the concentration of these ions

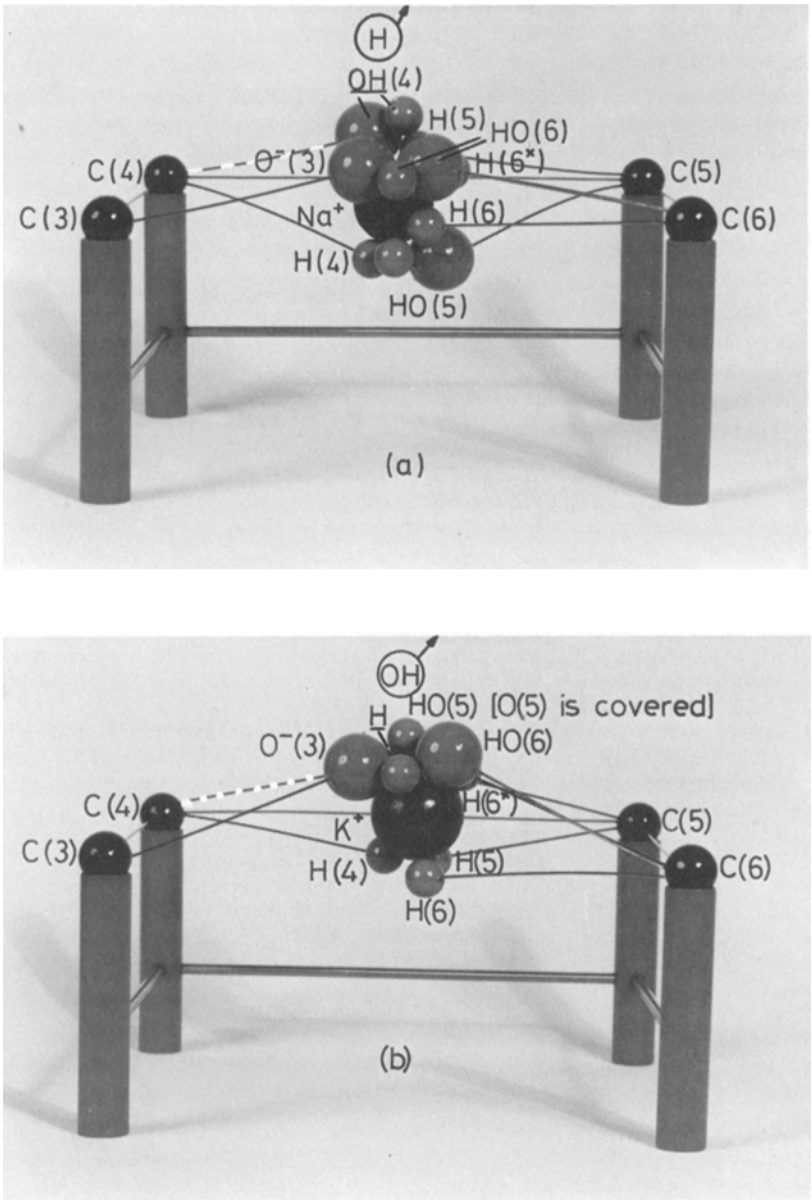

Fig. 2. Simplified model suggested for Na-ascorbate **(a)** and K-isoascorbate **(b)**. ———H (or OH) represent H or OH of water tightly bound to C(4). The radical is formed by removal of Ⓗ or ⓄⒽ of the tightly bound water. Since Na^+ and K^+ are considerably larger, the angles between the atoms above and below the plane determined by C(3)–C(4)–C(5)–C(6), e.g., between H(6) and HO(6), are actually about 140°. Furthermore, the angles between C(3)–C(4)–C(5) and C(4)–C(5)–C(6) are not rectangular but actually 116° and 110°, respectively

within the erythrocytes without detecting any change in the plasma in some diseases.

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