Note

Autoxidation Reaction Mechanism for L-Ascorbic Acid-related Compounds in Methanol without Metal Ion Catalysis

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The autoxidation mechanism for L-ascorbic acid (ASA)-related compounds such as D-arabino-ascorbic acid (=erythorbic acid; ERA) and triose reductone (TR) in methanol without metal ion catalysis was studied. The oxidation reaction of these ASA-related compounds seems to proceed via the C(2) oxygen adduct of ERA (or TR) by a similar reaction mechanism to that of ASA.

Key words: L-ascorbic acid; autoxidation; D-erythorbic acid; triose reductone; C(2) oxygen adduct

ASA is an important antioxidant in food and biological systems. It is known that ASA is easily oxidized to dehydro-ASA via monodehydro-ASA, but this oxidation reaction, especially the autoxidation mechanism, has not been fully clarified. We have investigated the autoxidation reaction mechanism for ASA and have previously reported that the autoxidation of ASA in methanol in the absence of metal ion catalysts led to the formation of L-threonolactone and oxalic acid (OXA), which were the same identified reaction products as those formed in the photooxygcnation of ASA, the oxidation reaction of ASA with singlet oxygen. These facts suggested the possible involvement of the C(2) oxygen adduct of ASA as an intermediate compound in this autoxidation process, and this postulation was supported by a semi-empirical molecular orbital calculation. We have also reported that the superoxide anion would be directly released from the C(2) oxygen adduct of ASA, forming monodehydro-ASA. In this study, the autoxidation mechanism for ASA-related compounds such as ERA and TR in methanol without metal ion catalysis was studied. ERA is an epimer of ASA, and TR is the simplest “aci-reductone”. Which has the same functional groups —CO—C(OH)—C(OH)— as ASA (Scheme 1). Separation and identification of the initial autoxidation products of ERA and TR were carried out, and the formation of the superoxide anion during the autoxidation of ERA and TR was examined. The semi-empirical molecular orbital calculation was also used to clarify the reaction mechanism.

Commercially obtained ERA was recrystallized and TR was prepared according to the method of von Euler et al. ERA and TR were dissolved in 200 ml of methanol, each at a concentration of 50 μM. Oxygen gas was bubbled through the solution via a glass filter at a flow rate of 200 ml/min for 30 to 90 min at 25℃. The remaining amounts of TR were determined by the UV method (at 275 nm). The oxidation reaction mixture of ERA was evaporated to dryness, and 0.5 ml of TMSI-H (hexamethyldisilazane-trimethylchlorosilane) or 0.25 ml of N-methyl-N-trimethylsilyl-trifluoracetamide (MSTFA) was added to the residue to prepare the TMS derivatives of the reaction products. These TMS derivatives were separated by GC and their structural analyses were performed by GC-MS under the same conditions as those described in previous papers. The oxidation products of TR were analyzed by HPLC with Shim-pack SCR-102H columns (300×8 mm i.d., Shimadzu) connected in series under the following conditions: mobile phase, 2 mm p-toluenesulfonic acid; flow rate, 0.8 ml/min; column temperature, 50℃; detection, conductometrically (reaction mixture, 2 mm p-toluenesulfonic acid containing 8 mm 2,2-bis(hydroxymethyl)-2,2’-dinitrolriethanol and 0.1 mm EDTA, Shimadzu CDD-6A). The superoxide anion generated in the oxidation reaction of ASA in methanol was detected with the nitro blue tetrazolium (NBT) reagent under the same conditions as those described previously. Molecular orbital (MO) calculations were done with a semi-empirical MO calculation program, MOPAC ver. 6.0, and PM3 hamiltonian was applied. The ratios of remaining ERA and TR were 77.8% and 71.6% at 30 min, and 45.9% and 42.7% at 60 min, respectively. They were not significantly different from those of ASA reported previously. In the gas chromatogram of TMS derivatives of the oxidation products of ERA in methanol, several peaks other than those of ERA and dehydro-ERA, the main oxidation product of ERA, were detected. Two of these peaks were identified as D-erythrulactone (ERL) and OXA by comparing their retention times with those of authentic samples.

![Scheme 1. Structures of ERA and TR.](image)
Fig. 1. Reduction of NBT in the Autoxidation of ERA and TR in Methanol. Each value is the mean ± SD (n = 6).

and their mass spectra with those of authentic ones reported in the literature. ERL and OXA would be formed via C(2)-C(3) bond cleavage of the C(2) oxygen adduct of ERA, in the same way as that of ASA. In the oxidation of TR in methanol, the formation of glyoxylic acid and formic acid, C(2)-C(3) fission products of the C(2) oxygen adduct of TR, was also confirmed by comparing their high-performance liquid chromatographic retention times with those of authentic compounds. The reduction of NBT during the oxidation of ERA and TR in methanol is shown in Fig. 1. The absorbance at 560 nm increased during the oxidation of ERA or TR, suggesting the formation of the superoxide anion in the autoxidation reaction of each of these compounds.

All these experimental results just described strongly suggested that these reactions might proceed via a similar autoxidation pathway to that of ASA\(^{3,4,7}\) and that the singlet oxygen might be involved in these reactions. Therefore, to get more information about the reaction mechanism, some semi-empirical MO calculations were made on the reaction of ERA with oxygen and that of TR with oxygen. The changes in the heat of formation of the super-molecule of ERA anion with singlet oxygen and that of TR anion with singlet oxygen are shown in Fig. 2. When an oxygen molecule approached the C(2) atom of the ERA anion, the heat of formation of the super-molecule of ERA anion with singlet oxygen tended to decrease (Fig. 2A), a similar result also being observed in the case of TR (Fig. 2B). Therefore, in the vicinity of ERA anion (or TR anion), it seemed that a triplet oxygen molecule might behave like a singlet oxygen. The heat of formation of the C(2) oxygen adducts of ERA and that of TR were −293.7 kcal/mol and −155.9 kcal/mol, respectively. Thus, the formation of the C(2) oxygen adduct was also suggested as an intermediate product during both the autoxidation of ERA and of TR. These calculated results seemed to support our experimental results already described.

Thus, the oxidation reaction of both ERA and TR

![Scheme 2. Possible Autoxidation Pathway for ERA and TR.](image-url)
seems to have proceeded via the C(2) oxygen adduct of 
ERA (or TR) by a similar reaction mechanism to that of 
ASA. The following reaction scheme for the autoxi-
dation of ASA-related compounds is suggested (Scheme 2). In this reaction scheme, when an oxygen molecule ap-
proaches very close to an ASA-related compound, 
triplet oxygen would behave like singlet oxygen, and the 
C(2) oxygen adduct would be formed, from whence two 
different pathways would be derived: 1) the superoxide 
anion would be formed generating the monodehydro 
form which would yield the dehydro form and ASA-
related compounds through disproportionation as has 
been seen in the autoxidation reaction of ASA; 2) 
without the release of the superoxide anion, C(2)–C(3) 
fission products such as ERL and OXA, glyoxylic acid 
and formic acid would be formed. All these facts sug-
gest that new oxidation pathways for ASA, which we 
have proposed earlier, might be operative in the oxida-
tion of other “aci-reductone” types of compound.

References

1) B. H. Bielski, in “Ascorbic Acid: Chemistry, Metabolism, and 
Uses,” ACS Advances in Chemistry Series No. 200, ed. by P. A.
Seib and B. M. Tolbert, American Chemical Society, 
2) M. Levine and K. Morita, Vitamins and Hormones, 42, 1-64 
(1985).
5) B.-M. Kwon and C. S. Foote, J. Am. Chem. Soc., 110, 6582-
6583 (1988).
61, 1693-1695 (1997).
(1955).
9) C. Beauchamp and I. Fridovich, Anal. Biochem., 44, 276-287 
(1971).
10) J. J. P. Stewart, MOPAC version 6.0; Frank J. Seiler Research 
Laboratory, U.S. Air Force Academy, Colorado 80840-6528, 
12) G. Petersson, O. Samuelson, K. Anjou, and J. von Sydow, Acta 
13) F. W. MacLafferty and D. B. Stauffer, in “The Wiley/NBS 
Registry of Mass Spectral Data,” vol. 2, John Wiley & Sons, 