ARTICLES

Oxidation of Ascorbic Acid in the Presence of Nitrites

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We have shown that ascorbic acid in neutral aqueous media rather rapidly interacts with nitrite ions to form dehydroascorbic acid. If the acidity of solution is sufficiently high (pH value does not exceed much above ~7) and the nitrite concentration is of order of 0.05 M or more, the only mechanism of the reaction under aerobic conditions includes, as the first, rate-limiting reaction step, the nitrosation of ascorbic acid. A second-order reaction kinetics was observed in respect to protons, which determined a sharp acceleration of the ascorbic acid conversion when the acidity of the medium was raised. Hence, a suggestion can be drawn that the presence of nitrites in vegetables under acidic storage strongly increases the decay of vitamin C.

Keywords: *Ascorbic acid; nitrite; oxidation; nitrosation*

INTRODUCTION

One of the most important problems in food ecology is the interaction of toxicants with vitamins. A fairly high content of nitrites in some vegetables raises a problem of possible negative influence of the nitrite ion on vitamins, primarily on vitamin C (ascorbic acid, AA). A discussion of this problem is urgent, taking into account the well-known ability of AA to undergo a rapid nitrosation in acidic media, *O*-nitrosyl-AA thereby being formed, producing unstable dehydroascorbic acid as the result of several subsequent rapid conversion steps (Dahn et al., 1960). The consecutive conversions starting from *O*-nitrosyl-AA is given in Scheme 1. As information on similar reactions in neutral media has not yet been published, we investigated the interaction of L-AA with nitrite ions in neutral and low-acidic media buffered with Tris-HCl and phosphate buffers.

EXPERIMENTAL PROCEDURES

L-Ascorbic acid was of "chemically pure" grade (Russian classification) and was used without further purification. Sodium nitrite, EDTA disodium salt (Trylon B), and phosphate buffer components were of the same quality. Tris(hydroxymethyl)aminomethane (Tris) was of an inferior purity and was used after the preliminary recrystallization from ethanol.

The rate of the AA conversion was measured at a temperature of 25 °C spectrophotometrically at a wavelength of 267 nm, where the reaction product did not absorb. The measurements were carried out on a SF-26 spectrophotometer equipped with a thermostating device. To reduce AA autoxidation induced by the traces of heavy metal ions (for instance, the content of these ions in disodium phosphate is up to 0.001%, and the content of lead in sodium nitrite up to 0.0005%), the activity of the latter was lowered by addition of EDTA. In each experiment, 17.5 μL of aqueous 5.2×10^{-3} M L-AA was added to 3 mL of thermostated 0.05–0.2 M sodium nitrite solution in 0.05 M Tris-HCl buffer, pH 6.7–7.3, or 0.067 M sodium phosphate buffer, pH 5.7–7.1 (all the pH values measured on a Soviet pH-340 apparatus at 25 °C with an accuracy 0.1 pH unit) with 5×10^{-5} M EDTA disodium salt. The concentration of dilute AA was 3×10^{-5} M. After addition of the AA solution to a buffer system, the drop in optical absorption at 267 nm was measured. In most cases, the rate

Scheme 1
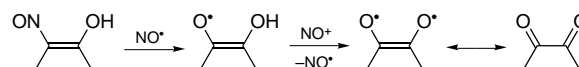
of the AA destruction was calculated as a reverse value of the reaction half-time. The final values of the above reaction rates were further calculated as an arithmetical mean of three runs. The experimental error did not exceed 5%.

In the next section, we shall also discuss some experiments beyond the main subject of the paper but of interest for the overall ecological problem.

RESULTS AND DISCUSSION

Despite complete binding of the trace heavy metal ions with EDTA, their catalytic activity for the AA autoxidation remained high enough. Because of this, the effect of nitrite addition to the AA solutions might be considered both as the result of the AA oxidation through a phase of O-nitrosation of AA and its autoxidation due to an increase in the solution ion strength or the formation of an AA–nitrite ion complex (Gumargalieva et al., 1989). An answer to this question, as the first approximation, might be obtained by means of the pH value variation. In such a way, we have established the period of the AA half-conversion ($t_{1/2}$) in 0.05 M Tris-HCl buffer in the absence of nitrite at pH 8.8 to be 58 min, at pH 7.3, 90 min, and at pH 6.7, 240 min. Such regularity fully corresponds to a decrease, with reducing pH value, in the concentration of the monoanionic form of AA (HA$^-$), which is more disposed to oxidation by molecular oxygen (Khan and Shukla, 1986). However, in the presence of 0.2 M NaNO$_2$, the $t_{1/2}$ value changes in a reverse order and is 30, 15, and 8 min, respectively. It means that, at pH 8.8, nitrite accelerates the reaction by 2 times whereas, at pH 6.7, the acceleration is 150 times. It is clear that the mechanism of the nitrite effect in the two cases should be essentially different. At pH 8.8, the acceleration of AA autoxidation upon the addition of nitrite may be considered the result of a nonspecific salt effect, while, at pH 6.7, the prevailing nitrite effect cannot be anything other than the result of an active specific interaction of nitrite with AA. This
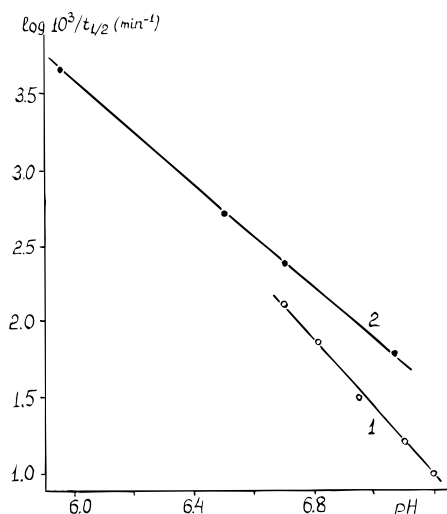
Ascorbic Acid Nitrite Oxidation

J. Agric. Food Chem., Vol. 44, No. 10, 1996 **2949**

Figure 1. pH dependence of the logarithm of AA oxidation rate (calculated as the reverse of the reaction half-time) with 0.2 M $NaNO_2$ and 5×10^{-5} M Trylon B at 25 °C in (1) 0.05 M Tris-HCl and (2) 0.067 M phosphate buffer. Initial AA concentration, 3×10^{-5} M.

Figure 2. Nitrosation−oxidation of AA in 0.05 M Tris-HCl buffer, pH 6.7, at 25 °C in the presence of varied amounts of $NaNO_2$: $[AA]_0 = 3 \times 10^{-5}$ M, [Trylon B] = 5×10^{-5} M, $\mu = 0.2$ M ($NaNO_2$ + $KClO_4$). (a) Kinetic curves: (1) 0.05, (2) 0.075, (3) 0.10, (4) 0.15, and (5) 0.2 M $NaNO_2$. (b) Logarithmic dependence of the AA oxidation rate on the nitrite concentration.

effect, in turn, is most probably O-nitrosation of AA followed by subsequent fast oxidation (Dahn et al., 1960). In such a case, at pH 7.3, the two types of AA oxidation would be competitive with each other.

We confirmed the above-made assumption in the following way. After blowing off the solution with argon to full deaeration, AA oxidation in the presence of 0.2 M $NaNO_2$ at pH 8.8 was virtually nonexistent; pH 6.7, blowing off O_2 did not affect the oxidation rate; and at pH 7.3, the observable rate of AA oxidation decreased by half. A more detailed experiment showed that, in 0.05 M Tris buffer and with $NaNO_2$ concentrations not lower than 0.05 M, pH 7.3 was really a boundary: at lower pH values, the AA oxidation through the nitrosation step was dominating whereas, at pH higher than 7.3, the contribution of the nitrosation−oxidation mechanism was small and became negligible with further increase in pH.

Information about AA autoxidation in neutral media is widely available (Khan and Martell, 1968; Hsieh and Harris, 1987; Yatsimirskii and Labuda, 1984). Due to this fact, the main subject of the discussion in the current paper will be that of data on AA oxidation in the presence of $NaNO_2$ at a pH lower than 7.3, that is, in the pH value region where the nitrosation−oxidation is a dominating pathway of the AA degradation.

Variation of the concentrations of AA, sodium nitrite, and protons within the pH range 6.7−7.3 made it possible to derive an approximate equation for the AA nitrosation−oxidation rate in Tris buffer under low-acidic pH values:

$$v = d[AA]^{0.3}[NO_2^-]^{1.25}[H^+]^2 \qquad (1)$$

The most important practical conclusion of the above equation is that the rate of AA decomposition in the presence of nitrite rises as the square of the proton concentration. It is well demonstrated by the dependence of log $v_{1/2} = 10^3/t_{1/2}$ (min^{-1}) upon the pH value as shown in Figure 1 (curve 1). The slope of this dependence is 2.16 ± 0.031, which approximates well enough to 2. A similar quantity obtained in phosphate buffer within the pH range 5.1−7.1 (Figure 1, curve 2) is 1.79 ± 0.04, which to some extent corroborates the previous result. Dependence of the rate of AA decomposition as
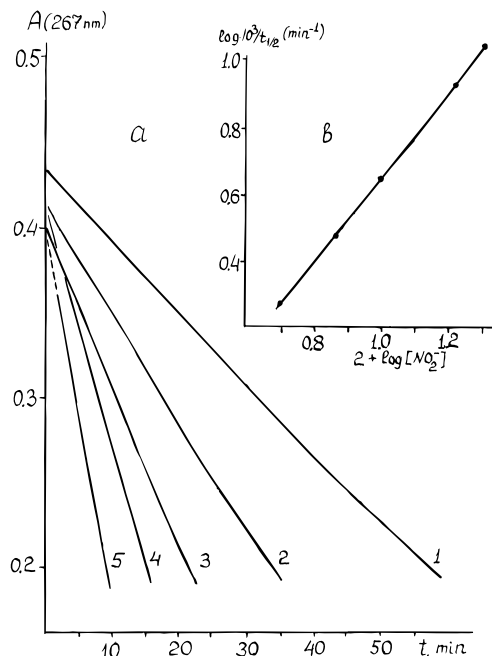
function of $[H^+]^2$ convincingly explains a rather sharp transition from the AA autoxidation catalyzed by nitrite to the nitrosation−oxidation of AA when passing from pH ∼7.3 to lower pH values. Indeed, in the pH region near 7.3, the rate of AA autoxidation undergoes a rather considerable drop with lowering pH value (by ∼2.5 times under the pH transition from 7.3 to 6.7), whereas the AA nitrosation−oxidation reaction is sharply accelerated.

A nitrite order of 1.25 is also of interest. In contrast to the proton order, whose magnitude steadily keeps close to 2, the nitrite order changes quite noticeably, depending on the medium composition. Particularly, at different pH values in the phosphate buffer , the nitrite order is close to 1.4−1.6. However, in both phosphate and Tris-HCl buffers, the nitrite order does not reach a magnitude of 2, which has been found in more acidic media (Dahn, 1960). Figure 2a represents a number of kinetic curves for the nitrosation−oxidation of AA in Tris buffer, pH 6.7 with varying $NaNO_2$ concentrations. The logarithmic dependence of the $v_{1/2}$ on the nitrite concentration (Figure 1b) allowed us to calculate the above-mentioned reaction order (precisely 1.23 ± 0.009) for nitrite ions. The most probable nitrosating agent−nitrous anhydride−however, formed according to reactions 2 and 3 (Ridd, 1961), requires the nitrite order to

$$HNO_2 + HNO_2 \rightleftharpoons N_2O_3 + H_2O \qquad (2)$$

$$H_2NO_2^+ + NO_2^- \rightleftharpoons N_2O_3 + H_2O \qquad (3)$$
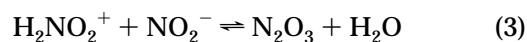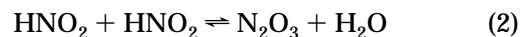
be 2. The nitrite first order might imply the nitrosating agent to be $H_2NO_2^+$, whose formation rate depends on $[H^+]$ and $[NO_2^-]$. Calculations were made, however, that showed the participation of $H_2NO_2^+$ in the limiting reaction step to be impossible for reasons both of very low equilibrium constant of the HNO_2 protonation and of very high rate constant of the same reaction. Besides,

2950 *J. Agric. Food Chem.,* Vol. 44, No. 10, 1996

Myshkin et al.

the proton second order suggests the nitrosating agent to be nitrous anhydride formed in reaction 2. So we cannot explain a low order for nitrite by simple kinetic reasons.

A rather low AA reaction order indicates that formation of a nitrosating agent is mostly the limiting step. Clearly, there should also be a concurrent reaction pathway in which AA is involved in the limiting step.

The kinetic data obtained make it possible to draw some practical conclusions as to a possible influence of nitrites on vegetables under storage conditions. Perform a rough calculation. According to our data, 0.83 mg/L AA in the presence of 9.2 g/L NO_2^- ions at pH 6.7 (Tris-HCl buffer) and a temperature of 25 °C decomposes by half in 1 min. Decrease now the concentration of NO_2^- ions to a reasonable practical value, for instance, to 10 mg/L. In this case, if nitrite order is equal to 1.25, the half-decay time of AA rises by $(9.2 \times 10^3/10)^{1.25}$ times and reaches ~5000 min, that is, close to 85 h. However, at rather slight acidification of the medium, even to pH 6.0, the AA half-decay time decreases by $(10^{-6}/2 \times 10^{-7})^2 = 25$ times and now amounts to ~3.5 h (at 5 °C ~25 h). At a higher acidity inside a vegetable, which is either specially created for storage or is endogenously developed during the storage, the time of AA half-decay at 25 °C can be reduced by tens and hundreds times: 100 times per each pH unit. Then it is possible to affirm that an elevated content of nitrate ions in vegetables and therefore correspondingly elevated content of nitrite ions produce a highly unfavorable medium for vitamin C under low-acid conditions. Naturally, our calculations are rather conventional and do not take into account many other chemical factors, which are determined by both the initial state of vegetable tissue and vegetable storage conditions. So our conclusions should be regarded only on a qualitative level. And still, as various chemical factors may not only inhibit the nitrite-induced AA destruction but, in some cases, also increase it (salt effects etc.), a danger of elevated AA destruction in the presence of nitrite ions must be taken into consideration. The following observation, in particular, could support the last statement: we have found that different nucleophiles (neutral bases and anions) may efficiently modify the nitrosating agent in accordance with eq 4

$$H_2NO_2^+ + B \rightleftharpoons NO^+B + H_2O \qquad (4)$$

Reagent NO^+B being more stable as compared to $H_2NO_2^+$ may be both less or more reactive than the latter.

A combination of factors of stability and reactivity of NO^+B is capable of considerably changing the rate of AA nitrosation.

The mechanism of AA nitrosation is more comprehensively discussed in our previous publications (Myshkin et al., 1990, 1991; Konyaeva et al., 1992).

LITERATURE CITED

Dahn, H.; Loewe, L.; Bunton, C. A. Uber die Oxidation von Askorbinsaure durch Salpetrigsaure. Teil VI: Ubersicht und Diskussion der Ergebnisse. *Helv. Chim. Acta* **1960**, *43*, 320–333.

Gumargalieva, K. Z.; Davydov, R.; Kalinina, I. G.; Moiseev, Yu. V. Nitrite and decomposition of ascorbic acid. *Zh. Fiz. Khim.* **1989**, *63*, 2539–2542.

Hsieh, I. P.; Harris, N. D. Oxidation of ascorbic acid by nitrite ion in copper-catalyzed sucrose solution. *J. Food Sci.* **1987**, *52*, 1384–1388.

Konyaeva, V. S.; Myshkin, A. E.; Gumargalieva, K. Z.; Moiseev, Yu. V. Nitrosation of ascorbic acid by nitrite ion in non-buffered neutral and moderately acid aqueous media. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1992**, (7), 1540–1544.

Myshkin, A. E.; Konyaeva, V. S.; Gumargalieva, K. Z.; Moiseev, Yu. V. Principal mechanisms of the oxidation of ascorbic acid in presence of nitrite in non-acid aerobic aqueous media. *Dokl. Akad. Nauk SSSR* **1990**, *315*, 383–387.

Myshkin, A. E.; Konyaeva, V. S.; Gumargalieva, K. Z.; Moiseev, Yu. V. Mechanism of nitrosation of ascorbic acid by nitrite in neutral aqueous media. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1991**, (10), 2242–2247.

Read, J. H. Nitrosation, diazotisation, and deamination. *Q. Rev.* **1961**, *15*, 418–441.

Taqui Khan, M. M.; Martell, A. E. Kinetics of metal ion and metal chelate catalyzed oxidation of ascorbic acid. III. Vanadyl ion catalyzed oxidation. *J. Am. Chem. Soc.* **1968**, *90*, 6011–6017.

Taqui Khan, M. M.; Shukla, R. S. Thermodynamics of the homogeneous oxidation of L-ascorbic acid by molecular oxygen catalyzed by ruthenium ion and ruthenium chelates. *J. Mol. Catal.* **1986**, *37*, 269–285.

Yatsimirskii, K. B.; Labuda, Ya. Oxidation of ascorbic acid by molecular oxygen catalyzed by tetrabenzo-tetraazamacrocyclic complex of copper. *Dokl. Akad. Nauk SSSR* **1984**, *276*, 880–883.