MECHANISM OF NITROSATION OF ASCORBIC ACID BY NITRITE IN NEUTRAL AQUEOUS MEDIA

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The main issues in nitrosation of ascorbic acid by the nitrite ion in aqueous media are discussed. Possible mechanisms of the reaction in aqueous media with different acidities are analyzed on the basis of available published data. The main kinetic characteristics of nitrosation of ascorbic acid in neutral Tris-HCl and phosphate buffers were obtained, and they are interpreted with due regard for the possible active participation of buffer components in the reaction.

Rather extensive publications [1-4] have been devoted to the mechanism of nitrosation of various classes of chemical compounds. Interest in these reactions is mainly due to the fact that the large-scale manufacture of one of the most important classes of dyes is based on one of the types of nitrosation, N-nitrosation of aromatic amines (diazotization). However, it cannot be said that other types of nitrosation, in particular, S- and O-nitrosation, have been investigated to such a degree. Publications [5-8] on these reactions indicate that the mechanism of nitrosation at S and O is multivariate and, in a number of cases, far from clear, which, by the way, can also be said of classical N-nitrosation. The purpose of the present paper is to approach an understanding of the mechanism of O-nitrosation of ascorbic acid (AA) because of the importance of the issue of the reaction of the nitrite ion with natural compounds.

L-Ascorbic acid (vitamin C), one of the most widespread and well-studied natural substances, exerts a strong reducing effect on a wide class of compounds. As a result of oxidation, AA is converted to dehydroascorbic acid, which is less stable than its reduced form [9]. The relatively facile degradation of dehydro-AA is the reason the oxidation of a significant amount of AA in a cell can lead to a significant decrease of the level of cellular vitamin C, which, in its turn, can seriously affect normal cell functioning. Together with natural metabolic processes accompanied by oxidation of AA, there can also occur various pathological processes due to penetration, into the cell, of various oxidizing agents or substances decomposing in the cell with the formation of agents actively oxidizing AA. The nitrite ion is one of such oxidizing agents, and, in a number of cases, the nitrate ion, which enters the cell in significantly large amounts, can be a precursor of the nitrite ion [10], because of which the issue of the toxicology of nitrates significantly intersects with the issue of the toxicology of nitrites.

The oxidizing effect of the nitrite ion with respect to AA is so well known that AA is even used as a reagent in determination of the concentration of nitrites [11, 12]. However, this reaction is recommended for use only in acidic and strongly acidic media, i.e., under conditions significantly different from the conditions characteristic of a living cell. Among the few investigations considering the mechanism of reaction of AA with nitrite, we should mention the papers of Bunton et al. [13, 14]. But Bunton et al. also restricted themselves to a study of only the acidic region with an upper limit of pH 5. However, this restriction does not lower the value of the conclusions that they obtained, which, we believe, are also significantly suitable for interpretation of possible mechanisms of the reaction of AA with nitrite under conditions of a living cell.

In [14], Dahn et al. proposed a mechanism in accordance with which the oxidizing effect of nitrite with respect to AA is due to a series of reactions limited by the first of them, O-nitrosation of AA in the 3 position. All subsequent reactions can be represented in the form of the simplified scheme presented below:

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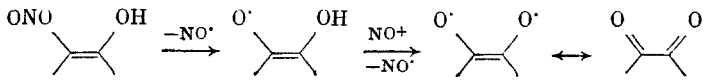


Because the oxidation of AA by nitrite is governed by the nitrosation of AA, in what follows, naming the entire series of reactions as a whole, we shall use the term nitrosation–oxidation of AA or, more briefly, nitrosation of AA.

The central issue that emerges in a study of the nitrosation of various compounds by nitrite is an effective nitrosation agent. Below we present the most important reactions of the nitrite ion in water [1]:

$$NO_2^- + H^+ \overset{K_1}{\rightleftarrows} HNO_2 \quad (1)$$

$$HNO_2 + HNO_2 \overset{K_2}{\rightleftarrows} N_2O_3 + H_2O \quad (2)$$

$$HNO_2 + H^+ \overset{K_3}{\rightleftarrows} H_2NO_2^+ \quad (3)$$

$$H_2NO_2^+ + NO_2^- \overset{K_4}{\rightleftarrows} N_2O_3 + H_2O \quad (4)$$

$$H_2NO_2^+ + B \overset{K_5}{\rightleftarrows} NO^+B + H_2O \quad (5)$$

(where B is a neutral or negatively charged base, which can be present in the aqueous medium).

Equations (1)-(5) indicate that in strongly acidic media one should expect a significant concentration of the nitrosacidium cation $H_2NO_2^+$ and the nitrous anhydride N_2O_3 resulting from it according to Eq. (4). It is logical to expect that in weakly acidic and neutral media the predominant nitrosation agent is N_2O_3, which forms according to Eq. (2) (it is assumed that NO_2^- and HNO_2 do not in themselves exhibit a significant nitrosation action [14]). However, the predominance or even significant predominance of N_2O_3 over $H_2NO_2^+$ under the given conditions cannot serve as the only argument in favor of the participation of only N_2O_3 in the nitrosation event. A significant role and in some cases a main role can be played by the significantly higher nitrosation activity of $H_2NO_2^+$ [1], which should probably be considered as a monohydrated nitrosyl cation where the water molecule is bonded in the NO^+ ion by an electrostatic bond rather than by a covalent bond [15]. These considerations suggest that even in media with relatively low acidity at rather high nitrite concentrations, which ensure an acceptable $H_2NO_2^+$ concentration, the nitrosation of AA can occur with predominant participation of $H_2NO_2^+$ or of its modification NO^+B, which is formed according to Eq. (5). Depending on the nature of the base B, both the stability of the compounds NO^+B and their nitrosation ability can vary in very wide ranges. In particular, it is known [16] that the constants of formation of complexes of NO^+ with thiourea, Cl^-, and Br^- are 5000, 32, and $5.1 \cdot 10^{-2}$ M^{-1}, and the rates of nitrosation of morpholine in the presence of these additives are correlated as 4200:240:1.

The present paper is devoted to an investigation of the kinetics of nitrosation of AA in neutral and weakly acidic buffer solutions.

EXPERIMENTAL

The main materials that were used were cp L-ascorbic acid, analytical purity-grade sodium nitrite, pure tris(hydroxymethyl)aminomethane (Tris) recrystallized from alcohol, and analytical-purity grade Trilon B (Czechoslovakia).

The reaction kinetics was measured spectrophotometrically with an SF-26 instrument. To 3 ml of a constant-temperature solution of sodium nitrite (0.05-0.2 M) with $5 \cdot 10^{-5}$ M Trilon B (for binding of metal ions catalyzing autooxidation of AA) in 0.05 M Tris-HCl (pH 6.7-7.3) or 0.065 M Na–K phosphate buffer (pH 5.9-7.0) was added 17.5 μl of $5.2 \cdot 10^{-3}$ M L-AA in water (the AA concentration after dilution was $3 \cdot 10^{-5}$ M), and the decrease of the absorbance at wavelength 267 nm, A (267), was measured with respect to time. In most experiments, the reaction rate was calculated as the reciprocal of half the reaction time: $v_{1/2} = 10^3/t_{1/2}$. In experiments with variation of the AA concentration, the arbitrary reaction rate was determined according to the slope of the middle part of the kinetic curves (the change of A with respect to t), the shape of which (except for small initial and final regions) was close to linear. The reaction rate values were calculated as the arithmetic mean of three measured values.

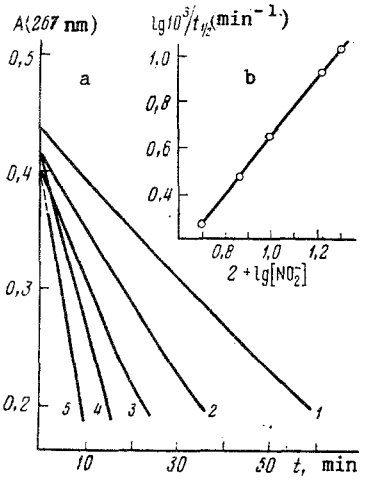


Fig. 1

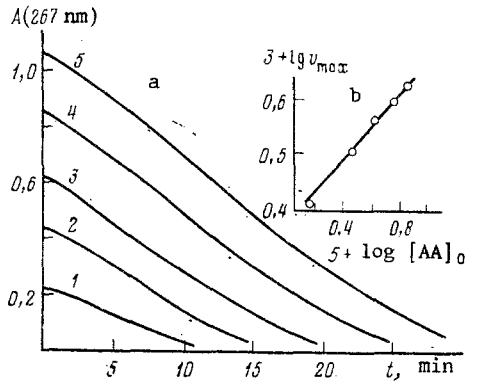


Fig. 2

Fig. 1. Nitrosation–oxidation of ascorbic acid in 0.05 M Tris-HCl buffer at pH 6.7 and 25°C in presence of variable amounts of $NaNO_2$. $[AA]_0 = 3 \cdot 10^5$ M, [Trilon B] = $5 \cdot 10^{-5}$ M, μ = 0.2 M ($NaNO_2$ + $KClO_4$): a) kinetic curves: 1) 0.05; 2) 0.075; 3) 0.10; 4) 0.15; 5) 0.2 M $NaNO_2$; b) logarithmic relation of rate of oxidation of AA to nitrate concentration.

Fig. 2. Nitrosation–oxidation of ascorbic acid in 0.05 M Tris-HCl buffer at pH 6.7 and 25°C with 0.2 M $NaNO_2$ with variation of initial AA concentration. [Trilon B] = $5 \cdot 10^{-5}$ M: a) kinetic curves: 1) $1.5 \cdot 10^{-5}$; 2) $3 \cdot 10^{-5}$; 3) $4.5 \cdot 10^{-5}$; 4) $6 \cdot 10^{-5}$; 5) $7.5 \cdot 10^{-5}$ M AA; b) logarithmic relation of rate of oxidation of AA (according to maximum slope of kinetic curves) to its initial concentration.

RESULTS AND DISCUSSION

It was determined that in an aerobic 0.05 M buffer of Tris-HCl at sodium nitrite concentration 0.2 M, temperature 25°C, and pH > 7.3, AA is oxidized predominantly via a nitrosation step, and at pH 7.2-7.0 the contribution of autooxidation of AA to the overall rate of its oxidation can be essentially neglected. This made it possible to determine the orders of the nitrosation–oxidation reaction of AA at pH 7.2-6.6 without blowing of oxygen. The reaction order with respect to a proton n (H^+) was ~2.2. In the phosphate buffer at pH 7.0-5.9, the value was somewhat lower, ~1.8. With neglect of the nature of the buffer in a first approximation, the reaction order with respect to the proton in the pH range from 7.2 to 5.9 could be approximated as 2.

Determination of the reaction order with respect to the nitrite ion was less single-valued. Variation of the nitrite concentration from 0.05 to 0.2 M at pH 6.7 and 25°C made it possible to determine n (NO_2^-) as ~1.13 (~1.03 at 40°C). However, during variation of the nitrite concentration with compensation for the ionic strength to 0.02 M by sodium nitrate, we obtained n (NO_2^-) as ~1.42, and using potassium perchlorate we obtained n (NO_2^-) as ~1.25. In a phosphate buffer at various pH values with compensation for the ionic strength by nitrate, n (NO_2^-) changed in the range from 1.4 to 1.6. We assume that the most objective is the result with perchlorate because this anion is coordinated very weakly to NO^+ and therefore has a minimum effect on the mechanism of nitrosation of AA. The kinetic curves of this series and the relation of $\log v_{1/2}$ to $\log [NO_2^-]$ are shown in Fig. 1.

The order with respect to AA was determined at pH 6.7 and 25°C. Figure 2 shows the results obtained in the Tris buffer that made it possible to determine n (AA) as ~0.3. In the phosphate buffer, the order with respect to AA was ~0.22. The fractional order with respect to AA indicates that two mechanisms are competing, in one of which AA participates in the limiting step and in the other of which it does not. In this case, the equation for the rate of nitrosation of AA can be written as follows:

$$v = -d[AA]/dt = k_1[NO_2^-]^{m_1}[H^+]^2 + k_2[AA][NO_2^-]^{m_2}[H^+]^2, \quad (6)$$

where m_1 is close to 1.25 because the first term of the equation is predominant, and m_2 in principle can vary from 1 to 2. However, in any case, it is significant that the averaged order with respect to nitrite is close to 1 rather than to 2, as should be expected for neutral media. Formally, this corresponds to participation of the nitrosacidium cation in the limiting step, i.e., to its formation or conversion to another form (excluding N_2O_3, which presupposes a second order with respect to nitrite). To verify these possibilities, it is necessary to carry out tentative calculations.

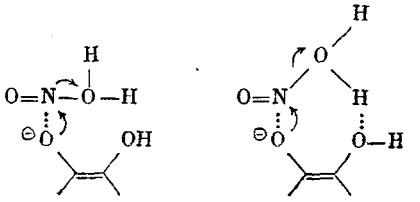
According to the data of [17], the constant of formation of $H_2NO_2^+$ from HNO_2 is $3\cdot10^{-7}$. This means, for example, that at pH 6.7 and $NaNO_2$ concentration 0.2 M the equilibrium concentration of $H_2NO_2^+$ in the solution will be $3\cdot10^{-7}\cdot0.2\cdot10^{-6.7}/7.1\cdot10^{-4} = 3.4\cdot10^{-7}$ M ($7.1\cdot10^{-4}$ is the equilibrium constant of dissociation of HNO_2). Because the order with respect to AA is close to zero, the rate constant of the reaction of the active form of the reagent with AA must be significantly greater than the observed nitrosation rate divided by the steady-state $H_2NO_2^+$ concentration and initial AA concentration. Taking into account the observed initial rate of nitrosation–oxidation of AA under these conditions at $[AA]_0 = 3\cdot10^{-5}$ M and 25°C is $\sim2\cdot10^{-8}$ M·sec^{-1}, we obtain $\sim2\cdot10^{13}$ M^{-1}·sec^{-1} as the value of the constant. But this value is two orders greater than the limiting value of the rate constants of the diffusion-controlled reactions in water ($\sim10^{11}$ M^{-1}·sec^{-1}). Therefore, the assumption that the kinetics of nitrosation of AA is limited by the conversion of $H_2NO_2^+$ should be rejected.

On the other hand, we also cannot accept to any greater degree the assumption that the kinetics is limited by the formation of $H_2NO_2^+$ as a result of proton transfer from H_3O^+ to HNO_2. According to the data of [16], the rate constant of such a reaction is $\sim5\cdot10^3$ M^{-1}·sec^{-1}. Calculations shows that in this case the rate of nitrosation of AA would be two orders greater than the experimentally observed rate. It remains to assume that in buffer solutions the AA nitrosation kinetics is actively affected by the buffer components, which form, according to Eq. (5), the corresponding nitrosyl derivatives NO^+B (B is Tris and/or Cl^- and, to a lesser degree, phosphoric acid anions). In this case, with a sufficiently high concentration of the nucleophile B, we can expect fast equilibrium formation of NO^+B in concentrations significantly greater than the equilibrium concentration of $H_2NO_2^+$. Taking into account what has been said, we can assume as the working hypothesis that in the limiting step the ionization of NO^+B (the predominant reaction) and, in parallel, the reaction of NO^+B with AA and NO_2^- occur. We also cannot deny that EDTA ($5\cdot10^{-5}$ M), which is added to the system, exhibits some activity with respect to NO^+. To refine the concepts concerning the reaction mechanism, we need kinetic data for an aqueous medium not containing nucleophiles other than NO_2^- that are able to undergo complexation with the nitrosyl cation.

Thus, enumerating the main possible mechanisms of nitrosation of AA in a neutral or weakly acidic buffer solution, we shall restrict ourselves to an indication of the limiting step and the order with respect to the nitrite ion:

1. $NO^+B \rightarrow NO^+...B$ (first)
2. $NO^+B + HA^- \rightarrow HA{-}NO + B$ (first)
3. $2HNO_2 \rightarrow N_2O_3 + H_2O$ (second)
4. $HA^- + H_2NO_2^+ \rightarrow HA{-}NO + H_2O$ (second)
5. $NO^+B + NO_2^- \rightarrow N_2O_3 + B$ (second)

We also cannot preclude the possibility of a mixed mechanism in which there occur protonation of nitrous acid in a complex with the HA^- monoanion and subsequent decomposition of the protonated complex with formation of O-nitroso-AA in the limiting step. This mechanism is supported by the specific reaction of the nitrite ion with AA observed previously [18] by ^{13}C spectroscopy under conditions virtually precluding nitrosation of AA. The transition states of such a mechanism of nitrosation of AA can have the following structure:



Data on the kinetics of autooxidation of AA in the presence of the nitrite ion in neutral and alkaline Tris buffers are given in [19].

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