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Ascorbic Acid Mediated N-Dealkylation and N-Deoxygenation of *N,N*-Dimethylaniline *N*-Oxide

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Abstract: Ascorbic acid ($AscH^-$), commonly known as vitamin C, is an antioxidant and radical scavenger present in many human and animal tissues. There is much current interest in the protective roles of antioxidants against degenerative tissue damage associated with aging and chronic diseases. We demonstrate that ascorbic acid mediates the facile N-deoxygenation and N-dealkylation of *N,N*-dimethylaniline *N*-oxide (DMAO) at room temperature to produce *N,N*-dimethylaniline (DMA), *N*-methylaniline (MA), and formaldehyde, respectively. The relative rates of N-dealkylation and N-deoxygenation were found to be dependent on both DMAO and $AscH^-$ concentrations in the reaction medium, and N-deoxygenation was somewhat faster than N-dealkylation under standard reaction conditions. Quantitative analysis of the products from DMAO/$AscH^-$ reaction mixtures indicated that the rate of N-deoxygenation is identical to the rate of $AscH^-$ consumption for at least the first 60 min of the reaction. Trapping experiments with mercaptoethanol and *N,N,N′,N′*-tetramethylphenylenediamine (TMPD) provided evidence that the $AscH^-$-mediated N-deoxygenation and N-dealkylation of DMAO is initiated by a single electron transfer process. A chemical mechanism for the $AscH^-$-mediated N-deoxygenation and N-dealkylation of DMAO is proposed, which entails initial formation of a nitrogen cation radical and a subsequently-formed carbon-centered radical species. Steady state kinetic studies were carried out, and the kinetic results support the proposed mechanism. The possible physiological significance of these reactions with regard to the metabolism of xenobiotic aromatic amines as well as the biological role of vitamin C in normal and disease states is discussed.

Hepatic monooxygenase activity toward amines is of particular concern since mankind is increasingly subjected to numerous pharmacologically active nitrogen compounds and various nitrogen-containing industrial chemicals. The N-oxidation of the arylamine and arylamide moieties of such compounds is often an essential step in their conversion into the ultimate carcinogenic derivatives.[1-5] It is generally accepted that while N-oxidation of tertiary amines to *N*-oxides or primary and secondary amines to hydroxylamines is mainly a function of the hepatic flavomonooxygenases,[6-8] N-dealkylation or

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carbon hydroxylation of these amines is a function of the P_{450} class of enzymes.[6-10] Several enzymatic[11] and chemical systems[12-16] have been studied as possible models for *in vivo* catabolism of amine oxides and hydroxylamines.

Ascorbic acid ($AscH^-$), commonly known as vitamin C, is an antioxidant and radical scavenger which is widely distributed in aerobic organisms and is found in high concentrations in many human and animal tissues. $AscH^-$ is known to protect cellular components against oxidative damage by free radicals and oxidants[17] and has also been shown to inhibit formation of *N*-nitroso compounds from amines in the presence of inorganic nitrites.[18-21] Recently, there has been renewed interest in the roles that vitamin C and other antioxidants may play in aging and in a number of chronic diseases, such as cancer, heart disease, brain dysfunction, and AIDS. In this report we demonstrate that $AscH^-$ mediates efficient deoxygenation and dealkylation of *N,N*-dimethylaniline *N*-oxide (DMAO) at room temperature under mild conditions, and we propose a chemical mechanism for these ascorbate-mediated processes. Our findings may have relevance not only to the metabolism of arylamine xenobiotics but also to the biological role of vitamin C in normal and disease states.

Experimental Section

Materials. Ascorbic acid ($AscH^-$), 2-mercaptoethanol, *N,N*-dimethylaniline (DMA), *N*-methylaniline (MA), sodium octylsulfonate, and *N,N,N',N'*-tetramethylphenylenediamine (TMPD) were from Aldrich and further purified either by reduced pressure distillation or by recrystallization. Analytical grade acetonitrile and methanol were dried over activated 4 Å molecular sieves and distilled under Argon.

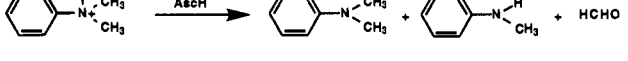
Methods. Synthesis of *N,N*-Dimethylaniline Oxide Hydrochloride. *N,N*-Dimethylaniline oxide hydrochloride was prepared by the oxidation of *N,N*-dimethylaniline with hydrogen peroxide in methanol according to the procedure by Shigeru et al.[22] Briefly, freshly distilled *N,N*-dimethylaniline (50 g) in 500 mL of methanol was treated dropwise with 350 mL of 30% H_2O_2. The reaction mixture was stirred at room temperature for 24 h, and excess H_2O_2 was decomposed with MnO_2 under cooling. The precipitate was filtered, and the filtrate was diluted with 3.5 volumes of water, washed with ether, and then acidified with concentrated HCl. The acidic solution was carefully evaporated under reduced pressure below 65 °C. The pale green crystalline residue was recrystallized from boiling acetone (41 g, 52%). Mp: 123-124 °C (lit.[22] mp 124-125 °C). ^{1}H-NMR (δ): 3.8 (s, 6H), 7.6 (m, 5H).

Reaction Conditions and Product Analyses. All nonaqueous anaerobic experiments were carried out under an atmosphere of argon in the presence of activated 4 Å molecular sieves with magnetic stirring at room temperature in dry solvents. The products of various reactions were analyzed by C_{18} reversed phase chromatography using 66% 0.1 M $NH_4H_2PO_4$ (pH 5.2) containing 5.0 mM sodium octylsulfonate, 22% acetonitrile, and 12% methanol as the mobile phase with UV detection at 254 nm. The products were quantitated by using standard curves of the respective authentic materials based on the peak heights. Whenever necessary, $AscH^-$ was quantitated by C_{18} reversed phase chromatography using 0.15 M monochloroacetic acid (pH 3.0) containing 2.0 mM EDTA and 0.1 mM sodium octylsulfonate as the mobile phase with electrochemical detection as described previously.[23,24] The formaldehyde product was identified and quantitated using the Nash reaction.[24] However, the precise quantitation of formaldehyde in various reaction mixtures was not possible due to instability of the Nash adduct under experimental conditions.[25]

Isolation and Characterization of the Products of the AscH/DMAO/2-Mercaptoethanol Reaction. Large-scale reactions (50 mM scale) were carried out under experimental conditions identical to those described above for small-scale reactions in the presence of equimolar concentrations of 2-mercaptoethanol in the incubation medium. The progress of the reaction was monitored by HPLC-UV. After all DMAO had been consumed the reaction mixtures were concentrated under reduced pressure, and products were separated by flash chromatography using 3% MeOH and 97% $CHCl_3$ as the eluent. The two low-polar products have been identified as DMA and the disulfide of mercaptoethanol by ^{1}H-NMR and by TLC comparison with authentic samples. The ^{1}H-NMR spectrum of the third product consisted of 7.25-6.6 (m, 5H), 4.6 (s, 2H), 3.7 (t, 2H), 3.0 (s, 3H), and 2.8 (t, 2H) δ in $CDCl_3$. This product was unstable and decomposed under basic conditions, yielding MA, formaldehyde, and 2-mercaptoethanol.

Results and Discussion

N,N-Dimethylaniline *N*-oxide hydrochloride, when incubated with $AscH^-$ at room temperature, readily decomposes to produce *N,N*-dimethylaniline and *N*-methylaniline as shown below.



The structures of these products were confirmed by isolation from large-scale reaction mixtures using preparative TLC followed by ^{1}H-NMR analysis. In addition, analysis of reaction mixtures for formaldehyde using the Nash reaction[24] revealed the formation of formaldehyde concomitant with N-dealkylation, as expected.[25] Formation of all three products (DMA, MA, and formaldehyde) is strictly dependent on the presence of $AscH^-$ and *independent* of the presence of molecular oxygen. Quantitative analysis of initial rates of product formation revealed that the apparent rate of N-deoxygenation is higher than that of N-dealkylation over a wide range of both $AscH^-$ and DMAO concentrations.

A typical time course for formation of DMA and MA when the reaction is run in anhydrous acetonitrile is shown in Figure

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(25) A typical reaction mixture containing 10 mM DMAO and 10 mM $AscH^-$ yielded approximately 1.0 mM MA and 2.4 mM DMA in a 60 min incubation period. When the same reaction mixture was analyzed using the Nash reaction for formaldehyde, the results indicated the presence of approximately 0.6 mM formaldehyde. However, the absorbance at 412 nm (λ_{max} of the Nash adduct) decays with time at room temperature (20% decrease in 10 min).

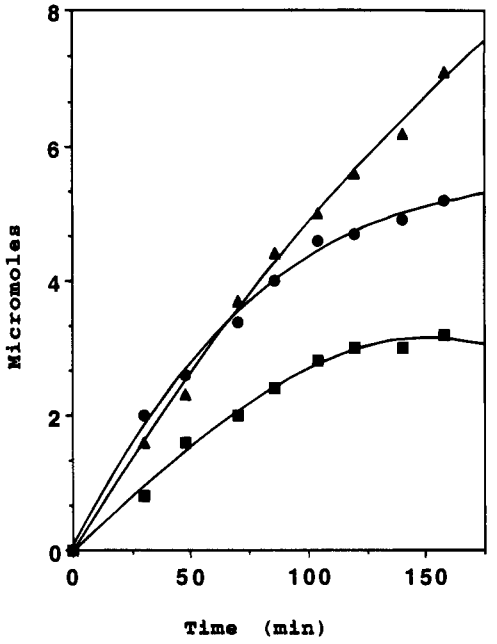


Figure 1. Time course of DMAO/AscH$^-$ reaction. A reaction mixture containing 16.0 mmol of DMAO and 17.0 mmol of AscH$^-$ in 10.0 mL of dry acetonitrile was stirred at room temperature under an atmosphere of Ar in the presence of 4 Å molecular sieves. At various time intervals, 10 mL samples of the reaction mixture were withdrawn and analyzed by C_{18} reversed phase HPLC for DMA, MA, and AscH$^-$ as described in the Experimental Section. The amount of AscH$^-$ consumed at each time point was calculated by subtracting the remaining concentration of AscH$^-$ in the reaction medium at a given time from the initial concentration: (▲) AscH$^-$ consumed; (●) DMA produced; (■) MA produced.

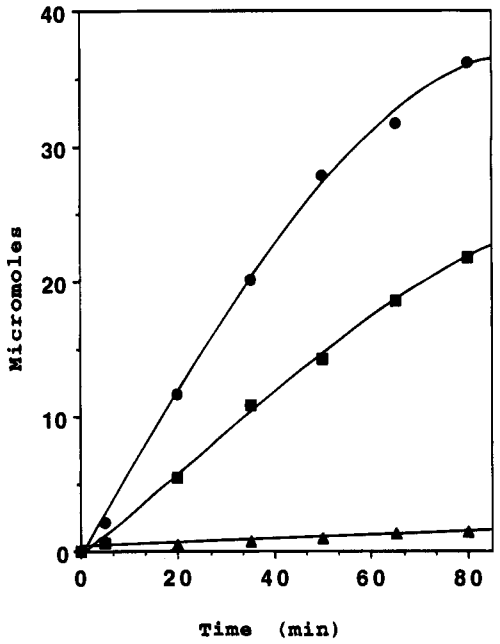
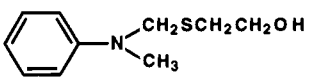


Figure 2. Effect of excess concentrations of 2-mercaptoethanol on the product distribution of the DMAO/AscH$^-$ reaction. The reaction mixture contained 57.0 mmol of AscH$^-$, 63.5 mmol of DMAO, and 146.0 mmol of 2-mercaptoethanol in 10 mL of dry acetonitrile. All the other conditions are identical to those described in the caption of Figure 1: (●) DMA; (■) new product; (▲) MA.

1. Also shown in the figure is the time course of AscH$^-$ consumption, as determined by HPLC with electrochemical detection using procedures which we have previously described.[23] It is evident from the figure that N-deoxygenation proceeds more rapidly than N-dealkylation, and that AscH$^-$ consumption is approximately stoichiometric with the amount of N-deoxygenation product, DMA, formed for at least the first 60 min of the reaction. In methanol, the rate of disappearance of DMAO is somewhat faster than in acetonitrile, whereas the reaction is much slower in 0.1 M NaOAC buffer, pH 5.2; in all cases, AscH$^-$-dependent decomposition of DMAO produces the same three products.

In order to determine whether the N-dealkylated product, MA, arises from dealkylation of DMA initially produced in the reaction mixture, the effect of exogenously added DMA on the rate of N-dealkylation was examined. A series of reactions was carried out with added DMA concentrations varying from 5 to 20 mM; in all cases the rate of N-dealkylation was not significantly affected by added DMA. Furthermore, inspection of reaction time courses revealed no significant initial lag period for either N-dealkylation or N-deoxygenation. These results indicate that the N-dealkylated product, MA, does not arise from direct dealkylation of DMA produced in the reaction mixture.

Since AscH$^-$ functions as a facile single electron donor in many chemical and biological systems, it seemed likely that the AscH$^-$-mediated decomposition of DMAO is initiated by a single electron transfer process giving rise to a nitrogen cation–radical, which could then undergo deprotonation at the α-carbon to form a carbon-centered radical species.[26] Therefore, we decided to explore the possibility of trapping reactive radical intermediates along the reaction pathway using thiol reagents.[27] When the AscH$^-$/DMAO reaction was carried out in the presence of 2-mercaptoethanol, the rate of N-deoxygenation was virtually unaffected, whereas the rate of N-dealkylation was considerably lowered. In addition, time-dependent formation of a new aromatic product was observed by HPLC using UV detection. The rate of formation of the new product is dependent on the concentration of 2-mercaptoethanol. It is evident from Figure 2 that, at an AscH$^-$/*N*-oxide/2-mercaptoethanol ratio of 1:1:3, formation of N-dealkylated product is virtually abolished, with only N-deoxygenation and formation of the new product being observable. Furthermore, in the absence of AscH$^-$, 2-mercaptoethanol itself is also capable of mediating the above reaction, producing only DMA and the new product. For the purpose of identification of the new product, large-scale reactions were carried out under standard experimental conditions in the presence of 2-mercaptoethanol. Three major products were isolated by flash chromatography. Two of these products were identified as DMA and the disulfide of 2-mercaptoethanol on the basis of their ^{1}H-NMR spectra. The third product was found to be relatively unstable, and it decomposed in the presence of 1 N NaOH to produce MA, formaldehyde, and 2-mercaptoethanol as identified by TLC and HPLC. On the basis of its ^{1}H-NMR spectrum (see Experimental Section) this new product was identified as the adduct of DMA and 2-mercaptoethanol, compound **1**. In particular, the presence of



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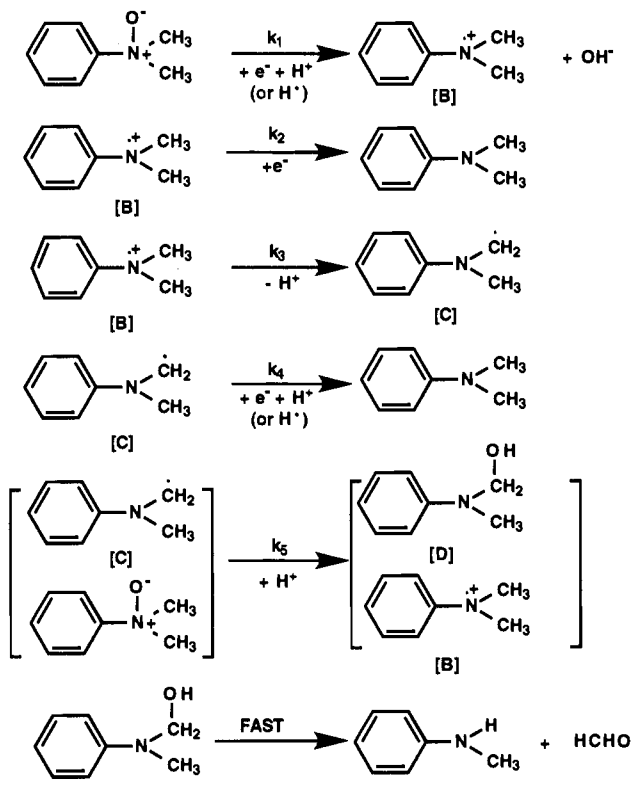
(27) Thiol reagents have been successfully used for trapping of radical intermediates in various organic reactions. For example, see: (a) Oswald, A. A.; Griesbaum, K. In *The Chemistry of Organic Sulfur Compounds*; Kharasch, N., Meyers, C. Y., Eds.; Pergamon Press: New York, 1966; Vol. 2, pp 233–256. (b) Erickson, R. E.; Holmquist, R. K. *Tetrahedron Lett.* **1969**, *48*, 4209–4212.

Scheme 1



a singlet at 4.6 δ and the lack of a second N−Me signal in the ^{1}H-NMR spectrum of the new product clearly indicate that this product is derived from coupling of the 2-mercaptoethanol moiety to one of the *N*-methyl groups of DMA.

If the decomposition of DMAO is indeed initiated by facile single electron transfer from AscH$^-$, it would be expected that DMAO should also be able to accept an electron from other single electron donors with appropriate reduction potentials. To test this possibility, we examined the ability of DMAO to accept an electron from *N,N,N',N'*-tetramethylphenylenediamine (TMPD), a single electron donor which has been very well-studied.[28-30] Upon addition of TMPD to a solution of DMAO under standard experimental conditions, the color of the reaction mixture immediately became intensely blue, a color change characteristic of formation of the TMPD cation radical. HPLC analysis of reaction mixtures with substoichiometric amounts of TMPD revealed time-dependent formation of both DMA and MA, with rates similar to those of corresponding AscH$^-$/DMAO reactions. In addition, neither the rate nor the product distribution of the AscH$^-$-mediated reaction was significantly affected by the presence of small amounts of TMPD. Thus, these results are consistent with the view that both N-dealkylated and N-deoxygenated products formed from DMAO are derived from a common intermediate generated via initial single electron transfer from either AscH$^-$ or TMPD.

Scheme 1 proposes a chemical mechanism for the AscH$^-$-mediated N-dealkylation and N-deoxygenation of DMAO which is consistent with all of these experimental observations. As shown, the mechanism indeed entails both initial single electron transfer to produce a nitrogen cation radical and subsequent formation of a carbon-centered radical, the latter being the species implicated by our mercaptoethanol trapping experiments.

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Additional aspects of the proposed mechanistic scheme are as follows: (a) The nitrogen cation radical, **B**, is common to both the N-dealkylation and N-deoxygenation processes. Species **B** is initially generated by single electron (or H atom) transfer from AscH$^-$ and homolytic cleavage of the N−O bond of DMAO. (b) The nitrogen cation radical, **B**, may undergo either radical termination, by abstracting a second electron to generate dimethylaniline, or deprotonation at the adjacent C−H moiety to generate the carbon-centered radical species, **C**. (c) The carbon-centered radical species, **C**, may simply abstract an electron plus a proton (equivalent to an H atom) to generate dimethylaniline. (Under our reaction conditions, both the k_2 and k_3 steps contribute to AscH$^-$-supported formation of the dimethylaniline product.[31]) Alternatively, species **C** may undergo a bimolecular homolytic oxygen transfer reaction with a *second* molecule of DMAO. This bimolecular reaction not only produces the carbinolamine species, **D**, but also *regenerates* the nitrogen cation radical species, **B**. (d) The final step in the dealkylation pathway is breakdown of the carbinolamine, **D**, to the N-methylaniline product. This is a very facile process which has been well-studied.[32]

In order to gain further support for the mechanism in Scheme 1, series of kinetic studies were carried out in which the concentration dependencies of the rates of N-deoxygenation and N-dealkylation were examined in detail. The results of these studies revealed that the initial rates of formation of either the N-deoxygenated product or the N-dealkylated product do not exhibit linear dependencies on the concentrations of AscH$^-$ or DMAO (reactants). Rather, it became clear that the ratio of initial rates of N-deoxygenation to N-dealkylation, at a constant AscH$^-$ concentration, is inversely proportional to the DMAO concentration, as shown in eq 1. As illustrated in Figure 3, a

$$\frac{\text{rate of N-deoxygenation}}{\text{rate of N-dealkylation}} = \frac{K}{[\text{DMAO}]} + C \qquad (1)$$

plot of the ratios of initial rates of N-deoxygenation to N-dealkylation vs 1/[DMAO] at a fixed AscH$^-$ concentration gives a straight line with a correlation coefficient of 0.99. It is also evident that both the slope and intercept of the plot are dependent on the initial concentration of AscH$^-$ present in the reaction mixture.

Steady state kinetic expressions were derived for the mechanism shown in Scheme 1 as well as two variations of this mechanism (see Appendix). The mechanism of Scheme 1 gives rise to the following expression for the ratio of initial rates of *N*-deoxygenation to *N*-dealkylation:

$$\frac{\text{rate of N-deoxygenation}}{\text{rate of N-dealkylation}} = \frac{[\text{AscH}^-](k_2k_4[\text{AscH}^-] + k_3k_4)}{[\text{DMAO}](k_3k_5)} + \frac{k_2[\text{AscH}^-]}{k_3} \qquad (2)$$

It is evident that eq 2 indeed predicts the results shown in Figure 3, i.e., the linear relationship between the initial rate ratio for

(31) On the basis of the steady state kinetic expression (eq 2) derived for Scheme 1, our experimental data give an average value for k_2/k_3 of 0.06 × 10^3 M^{-1} (from the *Y*-intercept in Figure 3). Therefore, since partitioning between step 2 and step 3 of Scheme 1 depends on the ratio k_2[AscH$^-$]/k_3, DMA formation occurs via both processes under our reaction conditions. We have observed that other electron donors (e.g., mercaptoethanol) apparently facilitate DMA formation via the direct reduction process of step 2.

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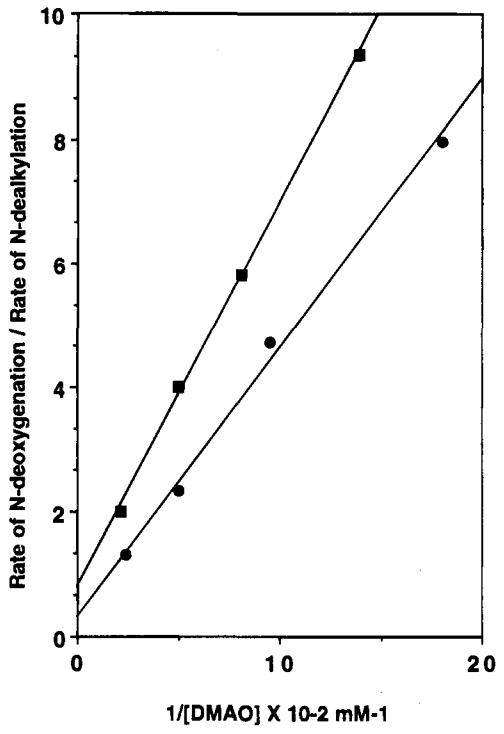

Figure 3. DMAO dependency of the relative rates of N-deoxygenation and N-dealkylation of the DMAO/AscH$^-$ reaction at a constant concentration of AscH$^-$. The initial rates of N-deoxygenation and N-dealkylation at a constant concentration of AscH$^-$ were measured by the HPLC–UV quantification of the products DMA and MA in standard DMAO/AscH$^-$ reaction mixtures as described in the Experimental Section except that anhydrous MeOH was used as the solvent: (■) 20 mM AscH$^-$; (●) 10 mM AscH$^-$.

the two reactions and 1/[DMAO], with AscH$^-$ dependencies for both the slope and the intercept. Thus, our kinetic results provide further support for the chemical mechanism outlined in Scheme 1.

The mechanism in Scheme 1 accounts for the striking difference in the nature of the ascorbate dependence between the N-deoxygenation and the N-dealkylation processes. Both N-deoxygenation and N-dealkylation are strictly dependent on the *presence* of AscH$^-$; in contrast, we find that N-deoxygenation, *but not N-dealkylation*, results in a net *consumption* of AscH$^-$. Inspection of Scheme 1 reveals that the proposed mechanism predicts precisely such a difference. Each N-deoxygenation event entails net consumption of an AscH$^-$ molecule, since two electrons are required for formation and collapse of the nitrogen cation radical species, **B**, to DMA product (steps 1–4). On the other hand, carbinolamine D, the immediate precursor of the N-dealkylation product, is produced in the bimolecular oxygen transfer reaction (step 5), which also regenerates species **B**. Thus, after initiation, the N-dealkylation cycle for DMAO proceeds without additional consumption of the electron donor, AscH$^-$.

Our results conclusively establish the ability of AscH$^-$ to carry out facile N-deoxygenation and N-dealkylation of DMAO under mild conditions. It is noteworthy that the mechanism we propose in Scheme 1 is reminiscent of the reaction mechanism which has been proposed for cytochrome P_{450}-catalyzed N-dealkylation of *N,N*-dimethylaniline.[9-10] However, it is important to emphasize that, unlike P_{450} catalysis, the ascorbate-mediated reaction we describe here does not require an external oxygen donor; DMAO itself serves as the sole oxygen source (see Scheme 1). TPPFe^{III}, a well-studied P_{450} model system, has also been reported to mediate the N-deoxygenation and N-dealkylation of DMAO in the absence of an additional oxygen donor,[33] but the relationship between this model and the physiological activities of P_{450} has been the subject of some debate.[11b] In this regard, we note that ascorbate is very widely distributed in aerobic organisms and is found in high concentrations in many organs, including kidney, liver, and brain as well as pituitary and adrenal glands.[34] For example, the ascorbate concentration in liver is 10–40 times that of plasma; the corresponding ratios are 40–125 for pituitary, 30–100 for adrenal, 13–38 for brain, 35–88 for leukocytes, and 5–38 for kidney.[35] Since the plasma ascorbate concentration is about 0.1 mM,[35] it is clear that ascorbate is present at millimolar concentrations, in many tissues. Thus, the AscH$^-$-mediated reactions described here could certainly represent physiologically relevant processes in various tissues.

As mentioned in the introduction, nitrogen-containing xenobiotics are primarily metabolized by two major microsomal monooxygenase systems. It is generally accepted that flavin-dependent monooxygenases (FMO) are mainly responsible for the conversion of aromatic amines to their corresponding *N*-oxides.[6-10] Once formed, these *N*-oxides can undergo the reactions of the ascorbate-mediated N-deoxygenation and N-dealkylation pathway described herein. It is evident from our results that, while the N-dealkylation branch of the pathway is autocatalytic after the initiation step, the N-deoxygenation branch is chain-terminating and consumes stoichiometric amounts of AscH$^-$. We have examined these reactions in a variety of aqueous and nonaqueous media. While the absolute reaction rates vary with solvent, we find consistently that N-deoxygenation predominates over N-dealkylation. Therefore, it seems likely that, in physiological environments, the radical-chain-terminating N-deoxygenation pathway will also predominate over the chain-propagating N-dealkylation pathway. If this were indeed the case, the result would be a cyclic metabolic process whereby an amine substrate would be initially converted to the corresponding *N*-oxide by FMO at the expense of NADPH, followed by regeneration of the amine through AscH$^-$-mediated N-deoxygenation of the *N*-oxide metabolite. We recognize, however, that such a scenario would probably require unusually elevated levels of *N*-oxide production before the extent of NADPH and AscH$^-$ consumption would represent a significant bioenergetic and nutritional burden to the organism. In this regard, it has been pointed out that ascorbic acid plays a significant role in regulating the concentrations and activities of both P_{450} and FMO,[34] and it is well-established that the levels of both of these enzymes are markedly reduced in vitamin C deficiency.[35] It is tempting to speculate that under conditions of ascorbate deficiency, the remaining minimal levels of this important vitamin are preserved by down-regulating the activities of these monooxygenases.

Ascorbate-mediated N-dealkylation and N-deoxygenation reactions such as those described here may be relevant not only to the metabolism of arylamine xenobiotics but also to the biological role of vitamin C in normal and disease states.

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Recently, there has been renewed interest in the roles that vitamin C and other antioxidants may play in aging and in a number of chronic diseases, such as cancer, heart disease, brain dysfunction, and AIDS.[37] Many lines of evidence point to the protective effects of antioxidants against damage to cellular components caused by reactive oxidants arising as byproducts of normal aerobic metabolism and respiration. Moreover, leukocytes and other phagocytic cells combat infectious agents by generating a mixture containing NO, O_2^-, and other reactive oxidants, and these oxidants may be at the root of pathological conditions associated with chronic infections.[37] It has been suggested that the diminished immune system function associated with chronic inflammatory diseases and with aging is at least partly due to the deleterious effects of reactive species such as NO on the proliferation of various lymphocyte subpopulations. This suggestion is supported by reports that both antioxidants and *N*-monomethyl-L-arginine, a competitive inhibitor of NO synthesis, at least partially alleviate these negative effects on lymphocytes.[37] The ascorbate-mediated N-deoxygenation chemistry described here may help define the biochemical processes which underlie these protective effects of antioxidants such as vitamin C.

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Appendix

Two variations of the chemical mechanism shown in Scheme 1 for the $AscH^-$-mediated N-dealkylation and N-deoxygenation of DMAO were considered.

1. *DMA is produced solely by one-electron reduction of the nitrogen cation radical* (species **B**). According to this mechanism, the carbon-centered radical species, **C**, readily undergoes the bimolecular oxygen transfer reaction with DMAO, but is not reduced to DMA (i.e., $k_4 = 0$). For this case, the steady state kinetic expressions for the ratio of initial rates of N-deoxygenation to N-dealkylation is as follows:

$$\frac{\text{rate of N-deoxygenation}}{\text{rate of N-dealkylation}} = \frac{k_2[\text{AscH}^-]}{k_3} \tag{3}$$

Equation 3 predicts that the initial rate ratio for the two reactions should be constant at a given ascorbate concentration. This is clearly inconsistent with the experimental results shown in Figure 3.

2. *DMA is produced solely by one-electron reduction of the carbon-centered radical* (species **C**). According to this mechanism, the nitrogen cation radical species, **B**, readily undergoes deprotonation at the adjacent C−H moiety to generate a carbon-centered radical, but is not directly reduced to DMA (i.e., $k_2 = 0$). For this case, the steady state kinetic expression for the ratio of initial rates of N-deoxygenation to N-dealkylation is as follows:

$$\frac{\text{rate of N-deoxygenation}}{\text{rate of N-dealkylation}} = \frac{k_4[\text{AscH}^-]}{k_5[\text{DMAO}]} \tag{4}$$

Equation 4 predicts that the ratio of initial rates of N-deoxygenation to N-dealkylation at constant ascorbate levels should be inversely proportional to [DMAO] with a zero intercept. This is inconsistent with our finding that both the slope and (nonzero) intercept of Figure 3 are dependent on the initial concentration of $AscH^-$ present in the reaction mixture.

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