Aerobic Oxidation of Aminoacetone, a Threonine Catabolite: Iron Catalysis and Coupled Iron Release from Ferritin

Fernando Dutra, Fernanda S. Knudsen, Denise Curi, and Etelvino J. H. Bechara*

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, CP 26077, 05513-970, São Paulo, SP, Brazil

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Aminoacetone (AA) is a threonine and glycine catabolite long known to accumulate in *cri-du-chat* and threoninemia syndromes and, more recently, implicated as a contributing source of methylglyoxal (MG) in diabetes mellitus. Oxidation of AA to MG, NH_4^+, and H_2O_2 has been reported to be catalyzed by a copper-dependent semicarbazide sensitive amine oxidase (SSAO) as well as by Cu(II) ions. We here study the mechanism of AA aerobic oxidation, in the presence and absence of iron ions, and coupled to iron release from ferritin. Aminoacetone (1−7 mM) autoxidizes in Chelex-treated phosphate buffer (pH 7.4) to yield stoichiometric amounts of MG and NH_4^+. Superoxide radical was shown to propagate this reaction as indicated by strong inhibition of oxygen uptake by superoxide dismutase (SOD) (1−50 units/mL; up to 90%) or semicarbazide (0.5−5 mM; up to 80%) and by EPR spin trapping studies with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), which detected the formation of the DMPO-$^{\bullet}$OH adduct as a decomposition product from the DMPO-$O_2^{\bullet-}$ adduct. Accordingly, oxygen uptake by AA is accelerated upon addition of xanthine/xanthine oxidase, a well-known enzymatic source of $O_2^{\bullet-}$ radicals. Under Fe(II)EDTA catalysis, SOD (<50 units/mL) had little effect on the oxygen uptake curve or on the EPR spectrum of AA/DMPO, which shows intense signals of the DMPO-$^{\bullet}$OH adduct and of a secondary carbon-centered DMPO adduct, attributable to the AA$^{\bullet}$ enoyl radical. In the presence of iron, simultaneous (two) electron transfer from both Fe(II) and AA to O_2, leading directly to H_2O_2 generation followed by the Fenton reaction is thought to take place. Aminoacetone was also found to induce dose-dependent Fe(II) release from horse spleen ferritin, putatively mediated by both $O_2^{\bullet-}$ and AA$^{\bullet}$ enoyl radicals, and the co-oxidation of added hemoglobin and myoglobin, which may be viewed as the initial step for potential further iron release. It is thus tempting to propose that AA, accumulated in the blood and other tissues of diabetics, besides being metabolized by SSAO, may release iron and undergo spontaneous and iron-catalyzed oxidation with production of reactive H_2O_2 and $O_2^{\bullet-}$, triggering pathological responses. It is noteworthy that noninsulin-dependent diabetes has been frequently associated with iron overload and oxidative stress.

Introduction

Aminoacetone is a threonine and glycine catabolite (*1*) long known to accumulate in the *cri-du-chat* (*2*) and threoninemia (*3*) syndromes and, more recently, pointed out as an alternative source of methylglyoxal (MG) to triose phosphate and acetone in diabetes mellitus (*4*). Threonine dehydrogenase catalyzes the oxidation of threonine by NAD^+ to glycine and acetyl-CoA (*5*), but when the ratio acetyl-CoA/CoA increases in nutritional deprivation (e.g., in diabetes) the enzyme produces AA (Scheme 1). In turn, AA as well as many other endogenous (e.g., methylamine) and xenobiotic amines (e.g., benzylamine) are oxidized by dioxygen in the presence of semicarbazide sensitive amine oxidases (SSAOs), a group of poorly understood plasma circulating and membrane bound Cu-dependent enzymes, yielding an aldehyde, H_2O_2 and NH_4^+ ions (*6*, *7*). With AA, SSAO activity paradoxally produces the cytotoxic and genotoxic MG (*8*, *9*).

Aminoacetone bears an amino group vicinal to the carbonyl function and therefore, like 5-aminolevulinic acid (ALA), a porphyrin precursor implicated in porphyric disorders (*10*), is expected to undergo phosphate-catalyzed enolization (*11*) and iron-catalyzed oxidation to yield reactive oxygen species (ROS), including $^{\bullet}$OH radicals (*12*−*16*). In this regard, Kawanishi et al. (*17*) recently reported that AA oxidation catalyzed by Cu(II) present in nucleus of human cultured cells is able to generate ROS and thereby promote DNA damage.

The present work aims to clarify the mechanisms by which AA undergoes direct and metal-catalyzed aerobic oxidation to yield deleterious ROS, with emphasis on the catalytic role of iron given its well-known implications in diabetes (*18*). We demonstrate here that the $O_2^{\bullet-}$ radical propagates oxygen oxidation of AA to iminoacetone, whose hydrolysis yields MG and NH_4^+ ions. We also demonstrate that simultaneous electron transfer

* To whom correspondence should be addressed. Phone: 55-11-3818-3869. E-mail: ebechara@iq.usp.br.

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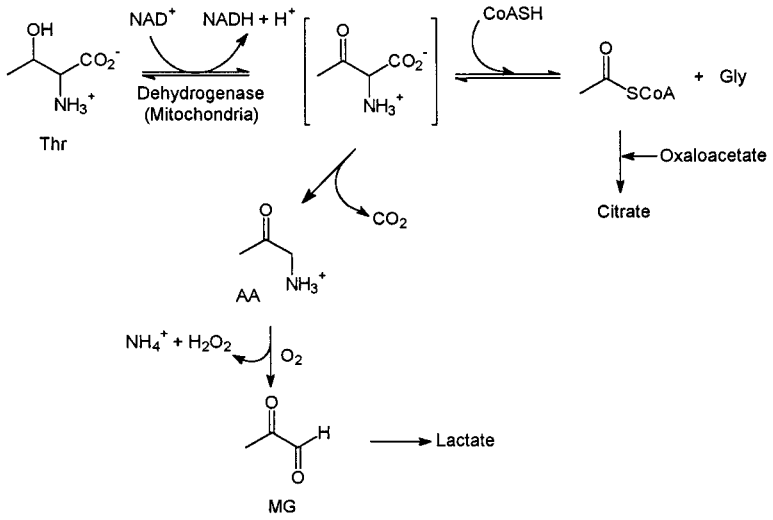
Scheme 1. Threonine Catabolism to Aminoacetone (AA) and Methylglyoxal (MG)



from both Fe(II) and AA to oxygen directly yields H_2O_2 and hence HO• radical (Fenton reaction), which accelerates the AA aerobic oxidation. Aminoacetone was also found to release iron from added horse spleen ferritin and to induce bovine hemoglobin (Hb) and myoglobin (Mb) one-electron oxidation to their *ferri* forms, pointing to a potential role of AA in impairing iron metabolism and triggering oxidative stress in diabetes and other maladies associated with AA overload such as threoninemia and *cri-du-chat* (*1*−*3*).

Materials and Methods

Reagents. All reagents, purchased from Sigma-Aldrich, were of purest analytical grade. Bovine oxy- and methemoglobin and oxymyoglobin were prepared and assayed as described elsewhere (*19*); their concentrations are expressed on a heme basis throughout this work. Horse spleen ferritin was purified on Sephadex G-25 as described by Oteiza et al. (*20*) to remove loosely bound iron. Ferritin concentration was determined by the Bradford method ([21]). Stock solutions of complexes of Fe(II), Fe(III), and Cu(II) with ATP, ethylenediaminetetraacetic acid (EDTA), and citrate were simply obtained by dissolving the correspondent salt in Milli-Q purified water containing ATP, EDTA, or citrate in a molar ratio of 1:1.2, and using the solution immediately after preparation. Stock solutions of AA (500 mM) were prepared in deaerated Milli-Q purified water. All buffers were pretreated with Chelex-100.

Aminoacetone Preparation and Product Analyses. Aminoacetone hydrochoride was prepared according to Hepworth (*22*) and recrystallyzed from ethanol:ether (8:2). Proton NMR analysis of AA (180 mM) was performed in a Varian T-200 spectrometer using dimethylsufoxide-d_6 (DMSO-d_6) or phosphate buffer prepared in D_2O (δ ppm, DMSO-d_6): 2.16 (3H, CH_3); 3.56 (2H, NH_2); 8.23 (2H, CH_2); δ (ppm, D_2O): 2.09 (3H, CH_3); 3.93 (2H, CH_2). NaOH voltametric titration of the α-ammonium group of AA hydrochloride resulted in a pK_a of 6.8 (see results). Aminoacetone semicarbazone was also prepared and recrystallyzed from aqueous ethanol to give colorless crystals (mp_{obs} 213 °C; $mp_{lit.}$ 212−214 °C) (*22*). Methylglyoxal was derivatized with 1,2-benzenediamine and analyzed by HPLC-diode array detection, a procedure adapted from Deng and Yu (*23*), as follows. Methylglyoxal was formed from the oxidation of 5 mM AA in air-equilibrated 100 mM phosphate (pH 7.4), at 37 °C, where dissolved oxygen is expected to be roughly 200 μM (*24*), in a flask with minimal headspace. After 2 h both perchloric acid (1 M), to stop the reaction, and *o*-phenylenediamine, to derivatize MG, were added to the spent reaction mixture (*23*). The mixture was heated for 3 h at 60 °C and the crude product analyzed by HPLC-diode array detection (at 315 nm). In turn, ammonia was assayed spectrophotometrically in the same final reaction mixture using a commercial kit (Ammonia Diagnostic Kit, Sigma) based on the NAD^+-dependent enzymatic amination of α-oxoglutarate.

Methods. Oxygen uptake was followed in a Yellow Spring Instruments Model 53 Oxygraph equipped with a Clark-type electrode. The oxygen consumption by AA (1−7 mM) was studied in 100 mM Chelex-treated phosphate buffer (pH 7.4), at 37 °C in the absence or presence of added metals. Iron release from ferritin was followed spectrophotometrically by the increase in absorbance at 530 nm due to the chelation of Fe(II) by bathophenanthroline sulfonate (ϵ_{530} 22.14 mM^{-1} cm^{-1}) (*20*). EPR spin-trapping with DMPO was studied at room temperature using a Brucker EMX spectrometer, 10 min after AA addition. The composition of the reaction mixtures and the operating conditions of the EPR spectrometer for each case are given in the legends to the figures. Aminoacetone-induced co-oxidation of oxyhemoglobin or oxymyoglobin (both 10 μM) in 10 mM Tris/glycine buffer (pH 7, 8) was followed by scanning the 500−700 nm region at selected time intervals or by monitoring the decay of absorbance at 577 and 582 nm, respectively, in a thermostatically controlled Hitachi U2000 spectrophotometer.

Results

Product Analysis. Like in the SSAO-catalyzed reaction (*25*, *26*), but differently in the Cu(II)-catalyzed oxidation (*17*), MG and NH_4^+ ions were found here to be formed from AA in stoichiometric amounts with the consumed oxygen: 205 ± 13 μM (triplicates) and 212 ± 15 μM (triplicates), respectively.

Oxygen Consumption. Oxygen depletion occurs within minutes upon addition of AA in Chelex-treated phosphate buffer following a typical autoxidation kinetics (Figure 1, line A). Maximum rates of oxygen uptake (k_{obs}, s^{-1}) respond linearly to the increase in AA concentration (3−20 mM) and allow the calculation of an apparent second-order rate constant, k_2, as 0.160 ± 0.007 M^{-1} s^{-1} (Figure 2). Neither Desferal (up to 3 mM) nor diethylenediaminepentaacetic acid (DTPA) (up to 3 mM) affect the AA oxidation rate (not shown), reassuring absence of contaminant metals in the buffer that are putatively able to catalyze autoxidation reactions (*27*). Addition of $O_2^{\bullet-}$ radical scavengers such as CuZn-superoxide dismutase (SOD) and semicarbazide (0.5−5 mM) (*28*) to the

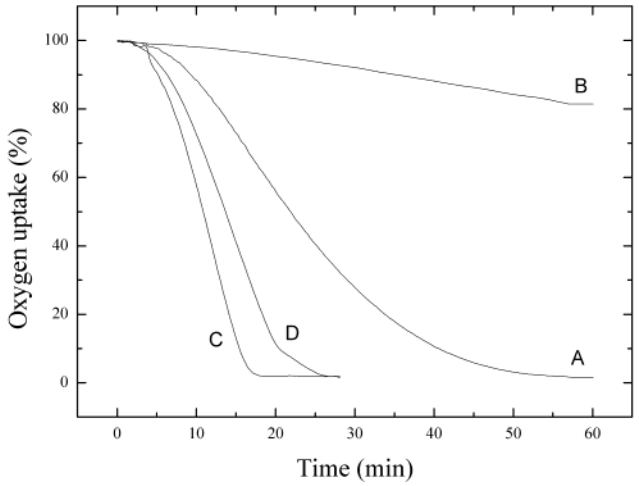


Figure 1. Effects of iron and SOD on oxygen consumption by aminoacetone. The time course of oxygen consumption by AA was monitored in 100 mM Chelex-treated phosphate buffer (pH 7.4), at 37 °C, containing: 5 mM AA alone, in the absence (control, line A) or presence of 50 units/mL SOD (line B), and with 30 μM Fe(II)EDTA in the absence (line C) or presence of 50 units/mL SOD (line D).

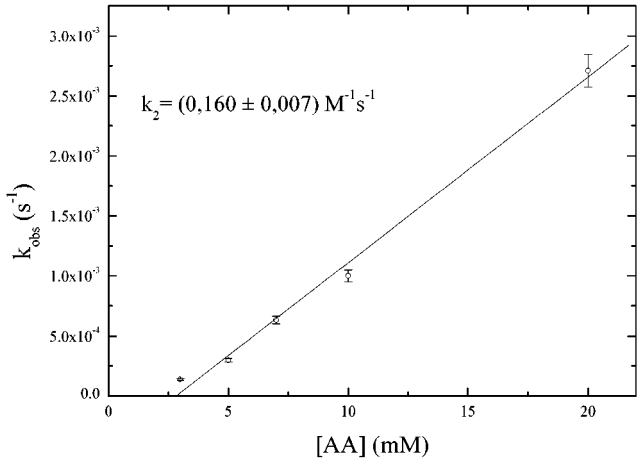


Figure 2. Effect of aminoacetone concentration on the observed autoxidation rate. Maximal rates of oxygen consumption by AA at increasing concentrations were measured in 100 mM Chelex-treated phosphate buffer (pH 7.4), at 37 °C and used to calculate k_{obs} and k_2 values, assuming $[O_2] = 200$ μM.

Table 1. Effect of Reactive Oxygen Species Scavengers on the Rate of Oxygen Uptake by Aminoacetone[a]

scavengers	metal	inhibition (%)
catalase	+Fe(II)	53
catalase	−Fe(II)	40
SOD	+Fe(II)	22
SOD	−Fe(II)	90
semicarbazide	±Fe(II)	80

[a] Oxygen uptake by 5 mM AA in 100 mM phosphate buffer (pH 7.4) at 37 °C, in the absence and presence of 30 μM Fe(II)EDTA. Catalase, 4.5 μM; SOD, 50 units/mL; semicarbazide, 5 mM. Inhibition values obtained from triplicates were reproducible within 15%.

reaction mixture inhibits oxygen uptake, thus evidencing the intermediacy of $O_2^{\bullet-}$ radical in the propagation step (Table 1 and Figure 1, line B). Furthermore, as in the case of the dihydroxyacetone phosphate autoxidation studied by Mashino and Fridovich (*29*), the ability of $O_2^{\bullet-}$ ions to abstract one electron from the substrate initiating the reaction is demonstrated here with the xanthine (50 μM)/xanthine oxidase (6 nM) system as a $O_2^{\bullet-}$ radical source (*30*) (Figure 3).

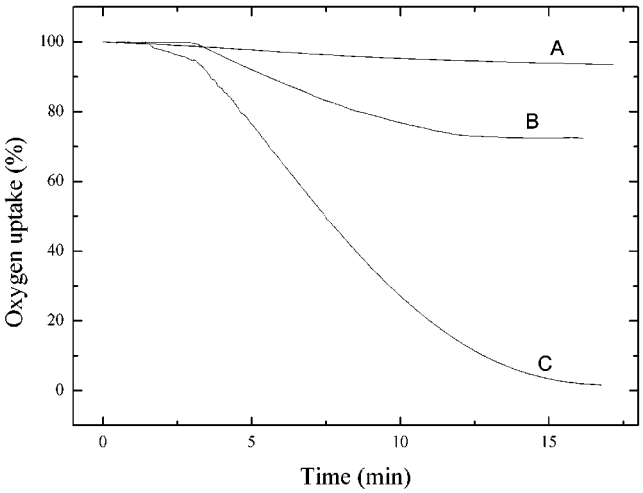


Figure 3. Cooxidation of aminoacetone with the xanthine oxidation reaction. The time course of oxygen consumption was monitored in 100 mM phosphate buffer (pH 7.4), at 37 °C, containing 1 mM AA (control, line A), the xanthine (50 μM)/xanthine oxidase (60 nM) system (control, line B) or the complete system (line C).

Table 2. Rate of Oxygen Uptake by Aminocetone in the Presence of Metal Complexes[a]

metal complexes (μM)	oxygen uptake rate (μM O_2/min)[b]
none	3.9
Fe(II)EDTA (30)	12.5
Fe(III)EDTA (30)	3.9
Fe(III)EDTA (200)	15.5
Fe(II)ATP (150)	5.0
Fe(III)ATP (150)	3.8
Fe(II)citrate (300)	5.3
Fe(III)citrate (300)	6.1

[a] Oxygen uptake by 5 mM AA in 100 mM phosphate buffer (pH 7.4) at 37 °C. Ratio chelator/metal = 1.2. [b] Observed rate values are reproducible within 15% (triplicates).

The Fe(II)EDTA complex (30 μM) was shown to accelerate the rate of oxygen consumption by AA (Figure 1, line C; Tables 1 and 2). Fe(III)EDTA and the Fe(II) and Fe(III) complexes with ATP or citrate were ineffective as catalysts in identical experimental conditions, although the former showed catalytic activity at a higher concentration (200 μM). For comparison, 50 μM $Cu(II)_{(aquo)}$ increased 20-fold the rate of oxygen uptake, whereas 100 μM Cu(II)EDTA has no effect. Interestingly, SOD (50 units/mL) had only a small inhibitory effect on the rate of oxygen uptake by AA in the presence of Fe(II)EDTA (Figure 1, line D; Table 1). This was interpreted as resulting from the prevalence of a metal-catalyzed two-electron reduction of oxygen to H_2O_2 and/or enzyme inactivation by a high flux of $HO^{\bullet}$ radical production (see spin-trapping studies below). Catalase (4.5 μM) addition to the reaction mixture decreased the observed rate of oxygen uptake by ca. 50% attesting stoichiometric formation of H_2O_2 (Table 1). One cannot exclude the possibility that H_2O_2 contributes to $HO^{\bullet}$ radical generation by the Fenton reaction, which is expected to oxidize AA directly and amplify the AA oxidation chain.

Oxidation of deprotonated AA similarly to ALA is preceded by its phosphate-catalyzed enolization (*12*, *31*), as indicated by the catalytic effect of phosphate on the rate of oxygen uptake by 5 mM AA [1.14 and 2.47 μM

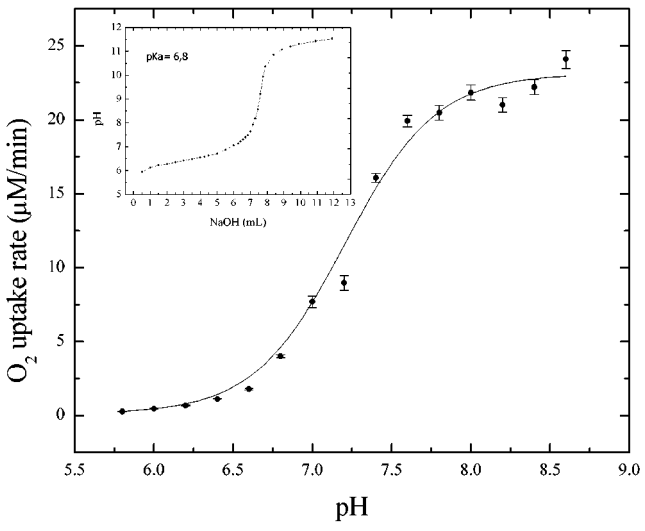


Figure 4. pH profile of aminoacetone iron-catalyzed oxidation. Oxygen consumption by 5 mM AA in the presence of 30 μM Fe(II)EDTA was monitored in 100 mM phosphate buffer, 37 °C, at differents pHs. The inset shows the titration curve of 5 mM AA with 100 mM NaOH in Milli-Q water.

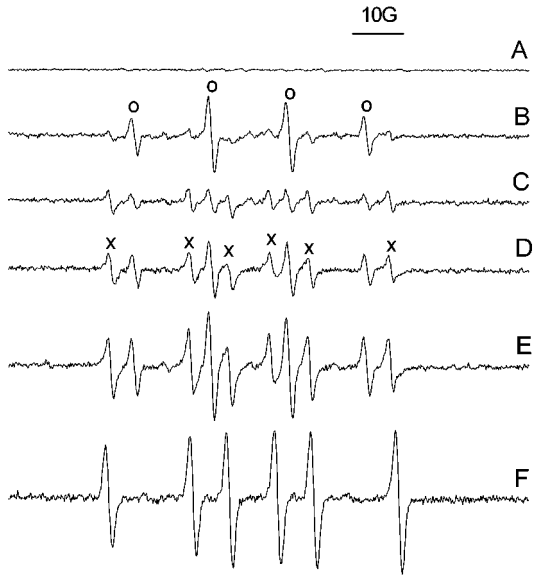


Figure 5. EPR spin-trapping studies of iron and SOD effects on the aerobic oxidation of AA. EPR spectra of DMPO-radical adducts were obtained after a 10-min incubation of 5 mM AA at 25 °C in 100 mM phosphate buffer (pH 7.4) with 100 mM DMPO: control without AA (A); plus 100 μM DTPA (B); plus 50 units/mL SOD and 100 μM DTPA (C); plus 30 μM Fe(II)-EDTA (D); plus 30 μM Fe(II)EDTA and 50 units/mL SOD (E); and plus 10% v/v DMSO and 30 μM Fe(II)EDTA (F). Instrumental conditions: microwave power, 20.2 mW; modulation amplitude, 1.0; time constant, 1.63 s; scan rate 0.1 G/s; receiver gain, 1.12×10^6.

O_2/min, at 50 and 100 mM phosphate (pH 7.4) respectively; not shown] and by the pH profile of the iron-catalyzed reaction showing an inflection at pH 7.2 (Figure 4), therefore slightly above the AA pK_a (6.8; inset Figure 4).

Epr Spin Trapping Studies. Figure 5 summarizes spin trapping studies conducted with AA (5 mM) in the presence of DMPO (100 mM) and both in the presence and absence of Fe(II). Hyperfine couplings were measured and radical adducts assigned from tables of known values (*32*). The observed 4-line spectrum B ($a_N = a_H =$ 14.6 G) of the AA/DMPO-containing reaction mixture is assignable to the DMPO-•OH adduct, formed by decomposition of the DMPO−$O_2^{\bullet-}$ adduct as suggested by the quenching effect of added SOD (50 units/mL) on the signal intensity (spectrum C); here, the presence of a 6-line signal attributable to a carbon-centered radical adduct (AA• enoyl radical?) is also revealed (compare with spectrum D). As DTPA (100 μM) addition has no effect on the spectra, confirming absence of adventitious iron in the Chelex-treated buffer and therefore excluding the occurrence of the Fenton reaction *strictu senso*. It is tempting to attribute •OH radical formation in the absence of iron to reduction of H_2O_2 by the hypothetical enoyl radical intermediate (AA•), similar to the so-called organic Fenton reaction with semiquinones (*33*).

Upon addition of Fe(II)EDTA, the spin-trapping experiments revealed two less intense spin adduct signals (spectrum D): a 6-line spectrum($a_N = 15.5$ G; $a_H = 23.2$ G) assignable to a DMPO adduct with a carbon-centered radical (hypothetical AA• intermediate), and the 4-line signal from the DMPO-•OH adduct. Consistent with the observed weak inhibitory effect of added SOD in the oxygen uptake curve (Figure 1), the spin trapping experiment with SOD in the presence of Fe(II)EDTA did not change the ratio of the 6-line and 4-line signals (spectrum E). The observed relative increase in •OH (and possibly AA•) formation in the presence of SOD may result from increased formation of H_2O_2 by concomitant two-electron transfer from AA and Fe(II) to O_2, followed by the Fenton reaction, relative to $O_2^{\bullet-}$-propagated oxidation. In fact, dimethyl sulfoxide (DMSO) addition to the reaction mixture caused replacement of the DMPO-•OH adduct with a DMPO-•CH_3 adduct ($a_H = 23.2$ G; $a_N = 15.9$ G) (spectrum F). All radical signals reported here were absent in control experiments without addition of AA (e.g., spectrum A), that is, those with DMPO−Fe(II)-EDTA alone and in the absence or presence of either SOD or DMSO.

Ferritin Iron Release. As in the case of ALA (*20*), we show here that $O_2^{\bullet-}$ and putative AA-derived enoyl radicals generated during AA oxidation are able to liberate Fe(II) from horse spleen ferritin (Figure 6). Control experiments show minor release of persistent loosely bound iron from the protein as previously reported when studying ALA (*20*). Superoxide dismutase addition partially inhibited Fe(II) release by 0.5 mM AA by 25% at 100 units/mL (Figure 6, line C), whereas nitrogen purging blocked the release (Figure 6, line D). These studies confirm the intermediacy of AA-generated $O_2^{\bullet-}$ and suggest the involvement of AA• radical in the iron liberation from ferritin like previously demonstrated with ALA (*34*). In turn, the results in the absence of oxygen show that AA itself cannot reduce ferritin ferric to ferrous ions.

Hemoglobin and Myoglobin Co-oxidation. As previously reported with ALA (*19*), addition of oxyHb or oxyMb (both at 10 μM) notably increased (10- and 8-fold, respectively) the rate of oxygen consumption by 5 mM AA, while undergoing co-oxidation to their ferri forms (Figure 7). MetHb formation is attested to by the decay of absorbance at 540 and 577 nm showing the well-defined isosbestic points at 522 and 587 nm reported elsewhere (*35*); metMb formation was monitored by the decay of absorbance at 543 and 582 nm. In both cases, the rate of oxyhemeprotein co-oxidation responds roughly linearly the AA concentration (0.5−5.0 mM; not shown) and the oxyHb oxidation could be inhibited by addition

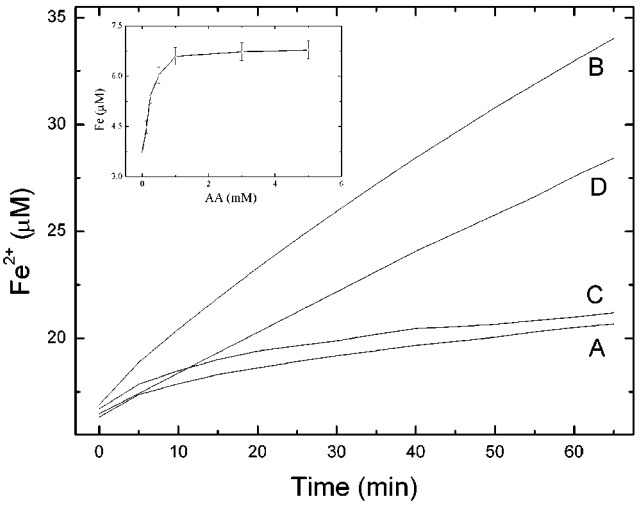


Figure 6. Time course of aminoacetone-induced iron release from ferritin. Iron release from ferritin (2.5 mg/mL) alone (line A) and in the presence of 0.5 mM AA (line B) was followed by measuring the increase in absorbance at 530 nm due to the chelation of Fe(II) by bathophenanthroline sulfonate. Experiments represented by lines C and D were performed in the presence of 100 units/mL SOD and upon nitrogen purging, respectively. Inset: Dependence of iron mobilization on AA concentration (0.05−5 mM) was measured after 30 min incubation in the standard buffer, at 37 °C.

of either 1.0 μM catalase (95%) or 60 units/mL SOD (13%), indicating a principal role for H_2O_2 via highly oxidizing $^{\bullet}OH$ radical and Hb-H_2O_2 complex in the oxyHb and AA co-oxidation.

Discussion

The present study demonstrates that AA undergoes autoxidation and iron-catalyzed oxidation propagated by $O_2^{\bullet-}$, which is accompanied by $HO^{\bullet}$ formation which, in turn, can amplify the chain reaction (Schemes 2 and 3). Methylglyoxal, NH_4^+ ions and H_2O_2 are the main oxidation products. The AA oxygen-consuming reaction is envisaged as preceded by phosphate-catalyzed AA enolization, like with ALA (*12*), followed by unielectronic reduction of oxygen by enol AA yielding a resonant enoyl radical ($AA^{\bullet}$) and $O_2^{\bullet-}$, which propagate the reaction. Hydrolysis of the oxidized AA, i.e., iminoacetone, results in MG and NH_4^+ ions, and $O_2^{\bullet-}$ dismutation yields H_2O_2, a source of $HO^{\bullet}$ radical by Fenton reactions with Fe(II) and $AA^{\bullet}$ radical. Accordingly, our data obtained by oxymetry and EPR spin-trapping experiments show that the reaction is inhibited by added SOD and by semicarbazide, both efficient $O_2^{\bullet-}$ scavengers. The same reaction mechanism has been shown to be operative in the case of ALA, a heme precursor which accumulates in some inherited (e.g., AIP) and acquired (e.g., lead poisoning) porphyric disorders and is able to provoke oxidative injury to several biomolecules and cell structures (*12*−*14*, *20*).

The small effect of SOD in the iron- and Hb-catalyzed AA oxidation, monitored by oxymetry and, in ther former case, also by EPR spin trapping, suggests prevalence of an $O_2^{\bullet-}$-independent pathway (Scheme 3): AA-Fe(II)-O_2 complexation, followed by simultaneous two-electron transfer from AA and Fe(II) to O_2 with direct H_2O_2 formation, a reaction analogous to an oxidation reaction studied by Caughey and collegues (*35*) with oxyhemoglobin, oxygen and nucleophiles. Further formation of $HO^{\bullet}$ radical from H_2O_2 plus Fe(II) and/or $AA^{\bullet}$ could amplify the chain reaction.

Aminoacetone is demonstrated here to induce Fe(II) release from isolated ferritin, an important biological iron storage site (*36*), promoted by reduction of Fe(III)-ferritin by AA-generated $O_2^{\bullet-}$ and $AA^{\bullet}$ radicals (Scheme 4). Our experiments run in the presence of SOD (10−400 units/mL) indicate that $O_2^{\bullet-}$ accounts for maximally 25% of the total iron mobilization (Figure 6), differently therefore to that observed with ALA (50% of released iron) (*20*). Enoyl $AA^{\bullet}$ radical like that formed by ALA oxidation is possibly responsible for the release of remaining iron. Indeed, during electrochemical one-electron oxidation of ALA in a nitrogen atmosphere (*34*), we were able to demonstrate the formation of a spin adduct with 3,5-dibromo-4-nitrosobenzene sulfonic acid (DBNBS), characterized by a 6-line signal, attributable to a resonant $ALA^{\bullet}$ enoyl radical. Iron release from ferritin added to this anaerobic system could be coupled to the electrochemical ALA oxidation, suggesting that $ALA^{\bullet}$ enoyl radical behaves like a semiquinone as an iron liberator from ferritin (*33*). In turn, hemoglobin and myoglobin catabolism are known to converge to hemin liberation and dioxygenation with further iron release, which according to Ryter and Tyrrell (*37*) could be involved in deleterious reactions that compete with iron reutilization and sequestration pathways.

The hypothesis involving AA oxidation by SSAO in diabetes-associated angiopathy (responsible for the typical nephropathy, neuropathy, and retinopathy) is consistent with the enzyme's predominance in the plasma membrane of vascular smooth muscle cells (*7*). However, the SSAO-catalyzed oxidation of AA as an exclusive route leading to MG and H_2O_2 fails to take into consideration that AA is not an ordinary amine, but an easily autoxidizable α-aminoketone, and that semicarbazide breaks $O_2^{\bullet-}$-dependent chain reactions. In addition, the low substrate specificity (several endogenous and synthetic mono- and polyamines) and high species-specificity of SSAOs, together with the production of MG, a highly cytotoxic and cross-linking agent, intrigues a physiological function for SSAOs (*7*).

To our knowledge, the physiological and pathological concentrations of AA in plasma and tissues have not yet been reported, perhaps due to its instability toward oxygen, especially in the presence of transition metal ions, and to the expected rapid Schiff conjugation of its oxidation product, MG, with proteins. Accordingly, (i) preparation of AA hydrochloride (*22*) requires strict dryness, glassware previously treated with metal chelators, and a nitrogen atmosphere, and (ii) the presence of AA in plasma has been inferred from the detection of a MG adduct with *o*-diaminobenzene. The only available in vivo AA estimation was long ago made by Urata and Granick (*38*), who found "about 0.4 mg of a compound with chromatographic properties of aminoacetone" in the urine collected daily from normal human adults, i.e., 2 μM AA assuming excretion of 1.5 L of urine/day. Therefore, the biological significance of our chemical studies with millimolar concentrations of AA should be viewed with perspective, although we have already demonstrated that swelling of isolated rat liver mitochondria can be attained with AA concentrations as low as 100 μM (work in progress).

Like ALA (also an α-aminoketone) (*12*) and simple sugars (α-hydroxycarbonyls) (*39*), AA undergoes fast

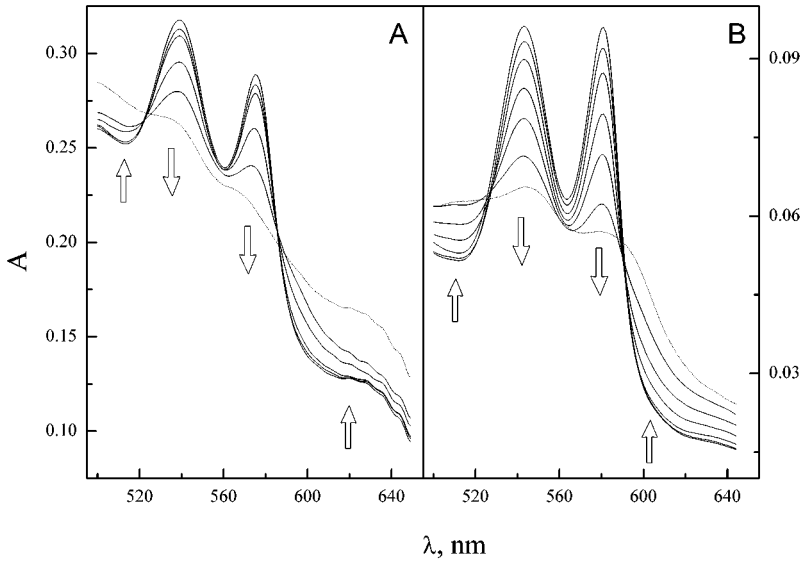
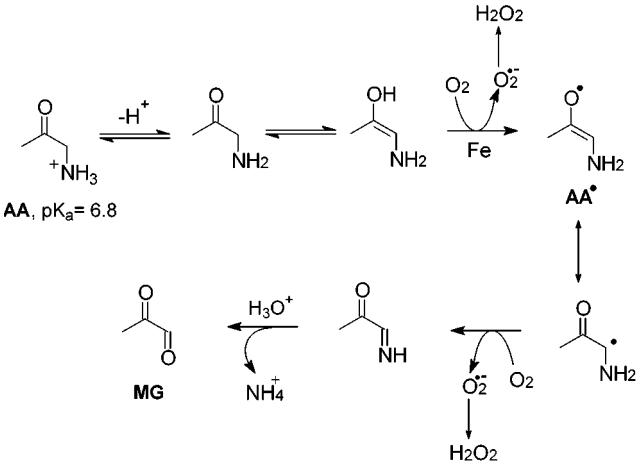
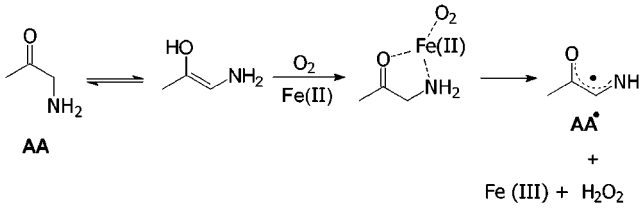


Figure 7. Cooxidation of aminoacetone and oxyhemeproteins. Temporal behavior of the spectra of oxyhemoglobin (10 μM, panel A) and oxymyoglobin (10 μM, panel B) in the presence of AA (5 mM) in 50 mM Tris-Gly buffer (pH 7,8) at 36 °C. OxyHb (A): 0, 10, 15, 20 and 25 min, and 60 min (broken line). OxyMb (B): 0, 5, 10, 15, 20 and 25 min, and 60 min (broken line). In the absence of AA, the absorption spectra underwent only very slight changes.

Scheme 2. Proposed Mechanism for the Aerobic Oxidation of Aminoacetone



Scheme 3. Catalytic Role of Fe(II) in the Aerobic Oxidation of Aminoacetone



$AA^{\bullet} + O_2 \longrightarrow AA_{imino} + O_2^{\bullet -}$

$Fe(III) + O_2^{\bullet -} \longrightarrow Fe(II) + O_2$

$Fe(II) + H_2O_2 \longrightarrow Fe(III) + HO^{\bullet} + HO^-$

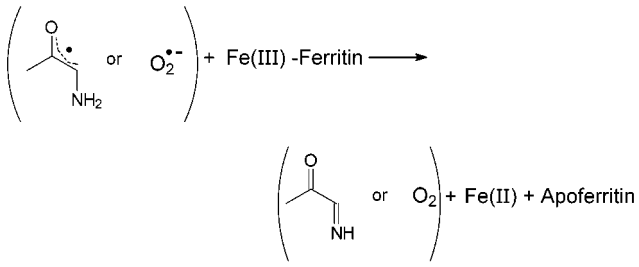
$AA + O_2^{\bullet -} \longrightarrow AA^{\bullet} + H_2O_2$

$AA + HO^{\bullet} \longrightarrow AA^{\bullet} + H_2O$

$AA_{imino} + H_3O^+ \longrightarrow MG + NH_4^+$

enolization at physiological pHs to give highly oxidizable enols, chemically similar to some extent to diphenols, aminophenols and diaminoaromatics. These compounds

Scheme 4. Ferritin Iron Liberation by $O_2^{\bullet -}$ and AA• Radicals



are also long known to accumulate in certain maladies and hence might act as efficient chemical sources of $O_2^{\bullet -}$ by autoxidation or metal-catalyzed oxidation (*40*, *41*). In this regard, noteworthy are (i) the reported "metal-free" oxidations of Amadori compounds prepared from fructose and Arg and Gly, found to be effective sources of $O_2^{\bullet -}$ by autoxidation of their enol forms (*42*) and (ii) the suggested implication of metal-catalyzed oxidation of glucose and glycated proteins yielding α-oxoaldehydes in protein damage in diabetes (*39*).

Several studies have suggested the role of iron in triggering oxidative stress and tissue damage in human and experimental animal diabetes (*18*, *43*, *44*). In advanced iron overload, known to be more effective than chronic liver disease and cirrhosis in generating diabetes, iron accumulation in pancreatic β-cells seems to impair insulin secretion, leading to insulin-dependent diabetes mellitus not reversible by iron removal. Accordingly, Okunade et al. (*45*) found significant high levels of nonheme iron and decreased reactive thiols in erythrocyte membranes of noninsulin-dependent diabetic patients, as compared to normal individuals, thus pointing to possible association of diabetes complications with iron-induced oxidative stress.

In conclusion, our studies point to AA as a potential source of MG and ROS by nonenzymatic aerobic oxidation and as an iron-releasing agent. The possibility raised here is that the chemical pathway of AA oxidation and iron liberation, in parallel to the reaction catalyzed by

SSAOs, may contribute significantly to oxidative stress triggered by diabetes.

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