Biochimica et Biophysica Acta 1840 (2014) 3190–3197

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Chemistry of free radicals produced by oxidation of endogenous α-aminoketones. A study of 5-aminolevulinic acid and α-aminoacetone by fast kinetics spectroscopy

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ARTICLE INFO

Article history:
Received 22 April 2014
Received in revised form 24 June 2014
Accepted 2 July 2014
Available online 10 July 2014

Keywords:
5-Aminolevulinic acid
α-Aminoacetone
Horse spleen ferritin
Human serum albumin
Antioxidants
Pulse radiolysis

ABSTRACT

Background: Excess 5-aminolevulinic acid (ALA) and α-aminoacetone (AA) are implicated in ketosis, porphyrinpathies and diabetes. Pathologic manifestations involve $^{\bullet}O_2^-$, H_2O_2, $^{\bullet}OH$, enoyl radicals ($^{\bullet}$ALA and $^{\bullet}$AA) and their oxidation end products.
Methods: To characterize enoyl radicals resulting from reaction of $^{\bullet}OH$ radicals with ALA and AA, micromolar $^{\bullet}OH$ concentrations were produced by pulse radiolysis of ALA and AA in aqueous solutions.
Results: ALA and AA react with $^{\bullet}OH$ at $k = 1.5 \times 10^9\ M^{-1}\ s^{-1}$. At pH 7.4, the $^{\bullet}$ALA absorbance spectrum has a maximum at 330 nm ($\varepsilon = 750\ M^{-1}\ cm^{-1}$). This band appears as a shoulder at pH 8.3 where two ALA species are present: $(NH_3)^+$-CH_2-CO-CH_2-CH_2-COO^- and NH_2-CH_2-CO-CH_2-CH_2-COO^- (pKa = 8.3). At pH 8.3, $^{\bullet}$ALA reacts with oxygen ($k = 1.4 \times 10^8\ M^{-1}\ s^{-1}$) but not with $^{\bullet}O_2^-$. At pH 8.3, AA oxidation produces two $^{\bullet}$AA species characterized by an absorbance spectrum with maxima at 330 and 450 nm. $^{\bullet}$ALA and $^{\bullet}$AA are repaired by antioxidants (quercetin (QH), catechin, trolox, ascorbate) which are semi-oxidized ($k > 10^8\ M^{-1}\ s^{-1}$). QH bound to HSA or to apoferritin and ferritin repairs $^{\bullet}$ALA and $^{\bullet}$AA. In O_2-saturated apoferritin solutions, $^{\bullet}$Q, $^{\bullet}O_2^-$, $^{\bullet}$AA and reaction product(s) react with QH.
Conclusions: The optical absorption properties and the time evolution of $^{\bullet}$ALA and $^{\bullet}$AA were established for the first time. These radicals and their reaction products may be neutralized by antioxidants free in solution or bound to proteins.
General significance: Adjuvant antioxidant administration may be of interest in pathologies related to excess ALA or AA production.

1. Introduction

The metabolism of amino acids in humans can be dramatically perturbed as a consequence of inborn or acquired enzyme deficiencies. As a consequence, accumulation of an essential metabolite among those involved in key metabolic pathways may occur. Such an imbalance can affect locally or in a systemic manner the function of essential organs. In this article, we focus on two α-aminoketones (AKs) involved in the normal metabolism of amino acids, namely, 5-aminolevulinic acid (ALA) and α-aminoacetone (AA) (see scheme 1). 5-Aminolevulinic acid is a heme precursor which accumulates in hepatic porphyrias and in

Abbreviations: apoFt, apoferritin; Ft, ferritin; SOD, superoxide dismutase; HSA, human serum albumin; AK, α-aminoketone; ALA, 5-aminolevulinic acid; AA, α-aminoacetone; G, radiolytic yield; AH^-, ascorbate; QH, quercetin; CatH, catechin; TrOH, trolox®

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http://dx.doi.org/10.1016/j.bbagen.2014.07.002
0304-4165/

tyrosinemia [1–3]. Excess ALA provokes acute neurological disorders and is implicated in a high incidence of hepatoma [4]. α-Aminoacetone is a threonine catabolite which accumulates in diseases characterized by high levels of circulating threonine such as threoninemia and the "cri-du-chat" syndrome [5]. Most importantly, excess AA is thought to be a source of methylglyoxal, a potent reagent of DNA bases and protein residues [3]. Both AKs are characterized by the presence of methylene groups adjacent to the carbonyl. α-Aminoacetone and ALA have one and two methylene groups, respectively. With such structural characteristics, these molecules undergo a rapid enolization catalyzed by phosphate ions at physiological pH. In the case of ALA, enolization occurs predominantly at C-5 [6]. These enols can donate one electron to oxygen by subsequent transition metal ion-catalyzed oxidation reactions in oxygenated media [3]. It has been proposed that, besides enoyl radical formation, these reactions produce superoxide radical anions and hence H_2O_2 which generate strongly reactive $^{\bullet}OH$ radicals by Fenton-like reactions [3,7].

It is therefore of interest to characterize the enoyl radical species ($^{\bullet}$ALA and $^{\bullet}$AA) formed by the reactions of $^{\bullet}OH$ radicals with ALA and AA. For these measurements, micromolar concentrations of $^{\bullet}OH$ radicals have been selectively produced by pulse radiolysis of buffered aqueous solutions of ALA and AA. Pulse radiolysis is a fast spectroscopic technique which provides spectral characterization as well as time-dependent behavior of radical species [8]. In N_2O-saturated solutions $^{\bullet}OH$ radicals can be produced alone although $^{\bullet}OH$ radicals and $^{\bullet}O_2^-$ radicals are produced simultaneously in O_2-saturated solutions. As the $^{\bullet}$ALA and $^{\bullet}$AA radicals are thought to play a role in the physiopathology of diseases mentioned above, it is important to examine the potential neutralization of these harmful radicals by antioxidants. Representative molecules from several families of antioxidants, namely, the hydrophobic flavone QH and the hydrophilic flavanol catechin (CatH), the vitamin E analog, trolox® (TrOH), and the ascorbate anion (AH^-) were selected for this model study.

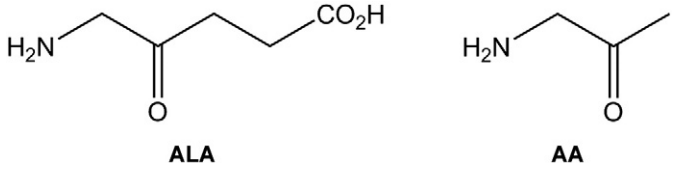
2. Materials and methods

2.1. Chemicals and routine equipment

All inorganic chemicals were of analytical grade and were used as received from the suppliers. 5-Aminolevulinic acid and AA were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Superoxide dismutase from bovine erythrocytes (SOD), horse spleen Ft and apoferritin (apoFt), delipidated human serum albumin (HSA), QH, CatH, TrOH and ascorbic acid were purchased from Sigma-Aldrich (St Louis, Mo, USA). Phosphate buffers were prepared in pure water obtained with a reverse osmosis system from Ser-A-Pure Co. The water from this system exhibited a resistivity of >18 MΩ cm^{-1} and a total organic content of <10 ppb. Absorption spectrophotometry was carried out in quartz cells with an Uvikon 922 spectrophotometer.

2.2. Pulse radiolysis

Pulse radiolysis measurements were performed with the Notre Dame Radiation Laboratory 8-MeV linear accelerator, which generates 5 ns pulses of up to 30 Gy. In general, the doses used in this work were ~20 Gy. The detection system, previously described [9,10], allows one to follow reaction kinetics in time domains up to 15 ms after the radiolytic pulse in the 290–700 nm spectral range. The radiolytic dose was determined using as a dosimeter the transient absorbance of the $^{\bullet}(SCN)_2^-$ radical-anion measured immediately after the radiolytic pulse at 472 nm. For this dosimetry (10^{-2} M SCN^- in N_2O-saturated solution), a radical chemical yield (G) × ε value for $^{\bullet}(SCN)_2^-$ equal to 5.28×10^{-4} m^2 J^{-1} was assumed. The G value is defined as the radical concentration divided by the radiolytic dose and can be expressed as μM Gy^{-1} (or μmol J^{-1} in dilute solution). The G value for $^{\bullet}OH$ in N_2O-saturated solution has been measured as ~0.64 μM Gy^{-1} [11]. Experiments were performed at room temperature (20 °C).



Scheme 1.

2.3. Preparation of the ALA and AA solutions for pulse radiolysis

The ALA and, to a lesser extent, AA rather readily auto-oxidize at mildly basic pH. Solutions (up to 5 mM) were therefore prepared in 0.1 M phosphate buffer (pH 8.3) at room temperature (20 °C) immediately before pulse radiolysis to avoid significant oxidation and/or dimerization (especially with ALA [6]) during the duration of a pulse radiolysis run (30 min or less). The pH drop (up to 1 unit), recorded after ALA solubilization in 0.1 M phosphate buffer (pH 8.8) was adjusted to pH 8.3 after stepwise addition of 1 M NaOH solution in water and the solutions were immediately saturated with pure N_2O. Some experiments were performed at pH 7.4 with 10 mM phosphate buffer.

2.4. Preparation of the Ft and apoFt solutions for pulse radiolysis

The Ft and apoFt stock solutions (48 mg mL^{-1}) from Sigma-Aldrich were delivered in 150 mM NaCl. Before pulse radiolysis measurements, these stock solutions were dialyzed twice against 1 L of pH 7.4 buffer. The dialyzed protein solutions were then diluted to the desired concentration assuming a molecular mass of ~440,000. An estimate of the Fe(III) content of Ft was obtained with the spectroscopic method developed by May and Fish [12] using the average value of the molar absorption coefficient given by Buettner et al. [13]. A solution containing 0.1 mg mL^{-1} leads to an absorbance of 0.23 at 380 nm. Thus, using ε(380 nm) = 19.0 ml $mg(Fe)^{-1}$ cm^{-1}, it was found that Ft contained ~1000 Fe(III) per molecule. Diluted solutions (up to 40 mL) were gently bubbled with the desired gas for 10 min prior to pulse radiolysis. To minimize the quantity of proteins consumed in each experiment, a micro-cell (optical path: 1 cm, volume: 120 μL) was used for transient recording. This micro-cell was emptied and refilled after each radiolytic pulse by a remote syringe pump to assure that all data were obtained with un-irradiated protein solutions. For the study of the effects of QH on protein radicals, 2 μM protein solutions were equilibrated for 2 h in the dark with aliquots of stock solutions of QH (10 mM in 0.1 M NaOH) to ensure full binding of the flavone to apoFt or Ft.

2.5. Analysis of kinetic data

Kinetics and spectral measurements were taken at least twice, and the results were found to be reproducible from day to day. Numerical integrations for analyses of complex rate data were carried out using Scientist software from Micromath Scientific Software. Rate constants were determined from raw data by the calculations with the above software.

3. Results and discussion

3.1. One-electron oxidation of AKs by $^{\bullet}OH$ radicals in N_2O- or O_2-saturated solutions

The sequence of reactions leading to the one-electron oxidation of AKs by $^{\bullet}OH$ radicals is as follows. The initial event is the radiolysis of water producing as main species: e_{aq}, $H^{\bullet}$, $^{\bullet}OH$, and H_2O_2. In N_2O-saturated solutions the hydrated electrons (e_{aq}) are converted into $^{\bullet}OH$ radicals by the reaction: $e_{aq} + N_2O + H_2O \dashrightarrow {}^{\bullet}OH + N_2 +$