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Original article

Quenching effect of deferoxamine on free radical-mediated photon production in luminol and *ortho*-phenanthroline-dependent chemiluminescence

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ABSTRACT

Removing excessive free radicals (FRs) by a synthetic chemical might give a clue for treatment of many iron-mediated diseases. Deferoxamine (DFO) can be one of the chemicals of choice for the clue. To investigate photoredox properties of DFO, its quenching effect on superoxide radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\bullet$) was examined using luminol and *ortho*-phenanthroline (*o*-phen) chemiluminescence (CL) systems and UV–vis spectrophotometry. Stern–Volmer equation was also used for the CL kinetics. The observed quenching effect of DFO on CL/photon production in luminol and *o*-phen CL systems strongly confirmed the static arm of quenching properties of DFO on $OH^\bullet$ and H_2O_2, but much less pronounced on $O_2^{-\bullet}$; the quenching property was maximal when iron was involved in the reaction systems. The Stern–Volmer plots in the designed photochemical reaction systems also confirmed a potent quenching effect of DFO on FR-mediated CL. Our study highlights strong photoreducing and antioxidant properties of DFO with huge quenching capacity on excessive FRs, and thus implies its promising prospects for therapeutic applications.

1. Introduction

Free radicals (FRs) such as $O_2^{-\bullet}$, H_2O_2, $OH^\bullet$, $^1O_2$ and $ONOO^-$ are normally generated *in vivo* [1–4]. Formed by one electron reduction of O_2, in the body, the $O_2^{-\bullet}$ is produced mostly in inflamed sites [1,2], dismuted to H_2O_2 [5,6] and further converted to $OH^\bullet$, mainly by Fe^{2+} and Cu^+, initiating Fenton's-like reactions and extensive oxidative damage to vital biomolecules like nucleic acids, proteins and lipids [7–9]. Among the FRs, $OH^\bullet$ highly reacts with functional groups of biomolecules and destroys them [10–12] (Eqs. (A) and (B)). Also, oxidation of Fe^{2+} by H_2O_2 produces $OH^\bullet$ [13]; the $OH^\bullet$ is an intermediate product of reactions in many biochemical systems such as, (A) $H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^\bullet + Fe^{3+}$, (B) $OH^\bullet + RH \rightarrow R^\bullet + H_2O$, (C) $R^\bullet + Fe^{3+} \rightarrow R^+ + Fe^{2+}$ and (D) $Fe^{2+} + OH^\bullet \rightarrow Fe^{3+} + OH^-$.

To combat the destructive effects of FRs, the body utilizes elaborate enzymatic/endogenous and non-enzymatic/exogenous antioxidant defenses [14] to quench or remove excessive FRs. Many synthetic chemicals also possess redox properties, eliminating oxidants–antioxidants imbalances *in vivo*. Among several available synthetic antioxidants, deferoxamine (DFO; Desferal®) can be a photochemical of choice for therapeutic purposes, and its clinical application in human and animal is promising [15–19].

As a siderophore, DFO is naturally produced by *Streptomyces pilosus*; it has been purified and synthesized since 1960 (Scheme 1A) [20]. As a specific iron chelator and by forming water soluble complex with iron, DFO effectively removes and eliminates excessive iron (Scheme 1B) [19,21], thereby balancing redox system in blood. Though to a much less extent than Fe^{3+}, DFO also exhibits affinity toward Al^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Ga^3 and other metal ions [22].

Despite its promising implication in medicinal chemistry, little studies have been done on photochemical properties of DFO in FRs producing chemical systems. This study aimed to pinpoint the luminescent properties of DFO to which how it behaves and interacts in the photochemical reactions systems using Fenton's

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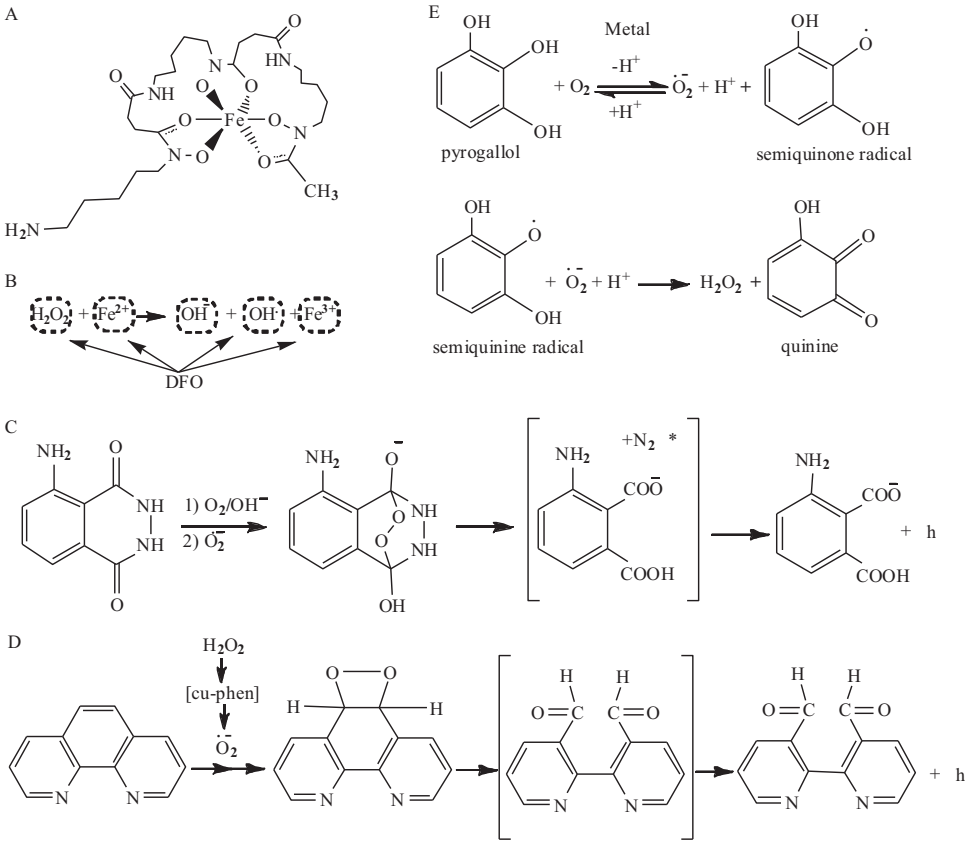

Scheme 1. Chemical structure of deferoxamine (DFO) in complex with iron (A) and its interaction with some free radicals in the Fenton's reaction system (B). Chemiluminescence (CL) mechanisms for luminol (C) and *ortho*-phenanthroline (D) and a generally accepted pyrogallol autoxidation pathway (E).

reaction and Fenton's-like reaction. To investigate photochemical properties of DFO, we tested the quenching and scavenging capacities of DFO on $OH^{\bullet}$, H_2O_2 and $O_2^{\bullet-}$ using luminol and *ortho*-phenanthroline (*o*-phen)-enhanced CL systems, UV–vis absorption spectroscopy and Stern–Volmer equation model.

2. Experimental

All chemicals and reagents were analytical grade. DFO, as mesylate salt (Desferal®), and *o*-phen were purchased from Sigma Chemical Co., St. Louis, MO, USA. Other chemical reagents were purchased from Merck, Darmstadt, Germany. Stock solutions of DFO (0.3 and 0.01 mmol/L in ddH_2O), luminol (0.1 mmol/L in DMSO, dimethylsulfoxide), *o*-phen (0.01 mmol/L in ddH_2O), $CuSO_4$ (0.01 mmol/L in ddH_2O), $FeSO_4$ (0.01 mmol/L in ddH_2O), were freshly prepared and appropriately protected from light for further use. Main buffers used in the study were phosphate-buffered saline solution (PBS), Tris–HCl, at pH 7.4 and 8, Tris–HCl at pH 8, carbonate at pH 10.2 and acetate at pH 5.5.

To test the effects of DFO on FR, various CL assays, in which the FR, especially $OH^{\bullet}$, H_2O_2 and $O_2^{\bullet-}$ that are central photo reactants *in situ*, were used. Photochemically, decrease of CL intensity in our method with DFO load always attributes to scavenging capacity/quenching ability of DFO on FR.

To examine the quenching effect of DFO on $OH^{\bullet}$-induced luminol CL, $OH^{\bullet}$ was generated by a Fenton's-type reaction [23] containing 100 μL $FeSO_4$ (0.4 mmol/L) and 100 μL of H_2O_2 1.5%. This mixture was incubated for 2 min at 37 °C and then 100 μL of PBS with and without different concentrations of DFO was added to the reaction mixture (solution 1). Luminol solution (600 μL of 0.15 mmol/L) was added into the luminometer cell (solution 2), and background of photon production was recorded on a FB12/Sirius Berthold ultra weak luminometer. Finally, 150 μL of solution 1 was added to the solution 2 and CL/photon production was counted (counts/10 s) and total CL count was integrated. Further, Stern–Volmer plot was drawn from equation $I_0/I = 1 + K_Q[Q]$ [24], where K_Q is the Stern–Volmer quenching constant, I_0 and I are CL intensity without and with DFO, respectively, and $[Q]$ is concentrations of DFO. Also % of scavenging capacity (SC) was calculated using: $SC = [(CL_{control} - CL_0) - (CL_{sample} - CL_0)]/(CL_{control} - CL_0)$, where $CL_{control}$ is the photon production of the control, CL_0 is the photon production of the background and CL_{sample} is the photon production of DFO mixed samples.

The inhibitory effect of DFO on Fenton's generated $OH^{\bullet}$ was performed using Cu^{2+} and ascorbic acid instead of Fe^{2+}, and *o*-phen was used as CL probe [25,26]. Briefly, 100 μL of 2×10^{-4} mmol/L $CuSO_4$, 100 μL of 10^{-3} mmol/L ascorbic acid, 100 μL of 10^{-3} mmol/L *o*-phen, 400 μL of 0.1 mmol/L acetate buffer and 100 μL of PBS with different concentrations of DFO. After recording the background CL (CL_0), the reaction was started after addition of 200 μL of 1 mmol/L H_2O_2. The CL intensity was counted once every 20 s at 37 °C. The Stern–Volmer quenching constant (K_Q) and SC were obtained as aforementioned procedure.

To evaluate the SC of DFO on H_2O_2, 600 μL of 50 mmol/L PBS, pH 8.0, with and without 200 μL of DFO in PBS and 200 μL of H_2O_2 1% were mixed for 10 min at 37 °C. Then 150 μL luminol (15 mmol/L) was added to the mixture; CL was quantified every 4 s, and KQ and SC were eventually obtained.

To examine the scavenging effect of DFO on $O_2^{-\bullet}$, the $O_2^{-\bullet}$ was generated from a pyrogallol autoxidation system accordingly [27]; the SC was determined with UV–vis spectrophotometer (cecill model 5000, Cambridge, England). Briefly, 500 μL of 100 mmol/L