Free Radical Research, Volume 38 Number 3 (March 2004), pp. 295–301

Taylor & Francis
healthsciences

Oxygen-Copper (II) Interplay in the Repair of Semi-Oxidized Urate by Quercetin Bound to Human Serum Albumin

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Accepted by Dr Michael Davies

(Received 13 May 2003; In revised form 1 September 2003)

The 1:1 complex of copper (II) and human serum albumin (HSA) slowly reacts with radiolytically generated $^{\cdot}O_2^-$ radical-anion at a rate constant of $6.1 \times 10^6\,M^{-1}\,s^{-1}$. Absorbance and fluorescence spectroscopies demonstrate that addition of an equimolar portion of quercetin (QH_2) to the solution of the copper (II)-HSA complex induces a relocalization of the copper resulting in a ternary copper (II)-QH_2-HSA complex. This form of quercetin slowly oxidizes in air-saturated solutions. A 10-fold excess urate, a plasma antioxidant, cannot displace copper (II) bound to HSA. In N_2O-saturated solutions the ternary complex form of QH_2 can repair the urate radical with a rate constant of $2.7 \times 10^6\,M^{-1}\,s^{-1}$ by an electron transfer reaction similar to that observed in the absence of copper (II). In O_2-saturated solutions and in the absence of copper, HSA-bound QH_2 fails to repair the urate radical because of the fast competitive reaction of $^{\cdot}O_2^-$ with urate radicals. However, addition of equimolar copper (II) restores the electron transfer from QH_2 to the urate radical. These contrasting results are tentatively explained either by an enhanced reactivity of copper (II) with $^{\cdot}O_2^-$ in the ternary complex or by direct production of quercetin radicals via a copper-catalyzed reduction of the $^{\cdot}O_2^-$ radicals by QH_2.

Keywords: Copper (II)-quercetin chelate; Pulse radiolysis; Absorption spectrophotometry; Fluorescence; Electron transfer; Superoxide radical-anion

INTRODUCTION

The oxidative modification of low-density lipoprotein (LDL) is currently thought to be a determinant of the atherogenic process.[1–3] It has been established that redox reactions induced by transition metal ions are involved in the oxidation of isolated LDL in buffered solutions.[4] Copper (II) ions have been shown to be the metal ions most effective in promoting both LDL lipid peroxidation and the oxidation of apolipoprotein B-100 residues such as Trp;[5] a process that, in turn, may induce LDL lipid peroxidation.[6]

However, under physiological conditions, several other factors must be taken into account when dealing with oxidative processes involving circulating molecules. First, most of the approximately 1 μg of copper per ml found in human plasma is bound to ceruloplasmin (93%) and is not exchangeable *in vivo*. The remaining copper is bound to human serum albumin (HSA).[7] At a physiologically relevant concentration, Cu^{2+} ions form tight 1:1 complexes involving His3 of HSA.[8] This latter fraction is in rapid equilibrium with tissue copper and is considered to be the intermediate form of copper transported in plasma.[7] Secondly, various antioxidants intervene in the oxidation of plasma constituents. Human plasma contains a high concentration of urate (about 0.3 mM), a product of purine metabolism. Due to the favorable redox potential of the urate radical/urate couple,[9–11] this molecule can function as a constitutive antioxidant,[9] by reacting with harmful oxygen radicals such as $^{\cdot}O_2^-$ or by repairing amino acid radicals of proteins.[12] Another important category of small

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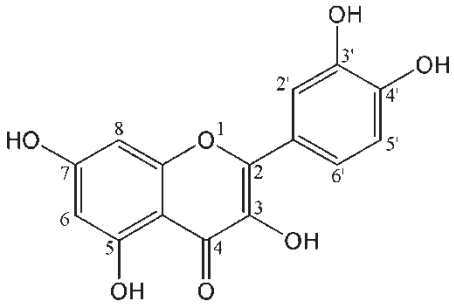
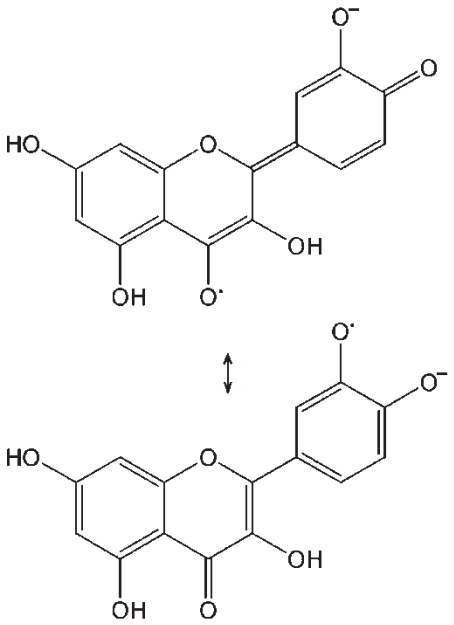
ISSN 1071-5762 print/ISSN 1029-2470 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/10715760310001626257

antioxidant molecules is the group introduced by dietary uptake such as vitamins C and E, carotenoids, and flavonoids. The flavonoids are plant compounds whose consumption has been associated with the protective effect of certain diets against some of the complications of atherosclerosis.[13] The antioxidant properties of flavonoids have been attributed to their scavenging of lipid alkoxyl and peroxyl radicals through hydrogen donation, to the regeneration of α-tocopherol by α-tocopheroxyl radical reduction and also to flavonoid chelation of transition metal ions.[14] Conjugates of several dietary phenolic compounds including catechin, quercetin and rutin have been found free in plasma or bound to HSA.[15,16] In a recent study of flavonoid-urate interplay related to copper induced oxidative stress in dilute plasma, we have demonstrated an antioxidant synergy between urate and each of the flavonoids: luteolin, rutin, catechin or quercetin. The flavonoids also protected endogenous urate from oxidative degradation.[17] However, using the pulse radiolysis technique, we have shown that in deaerated aqueous solutions, the urate radical resulting from the one-electron oxidation of urate is only partially repaired by HSA-bound quercetin[12] but not by catechin[11] or HSA-bound rutin.[12]

Considering all the above literature data on the status of urate and flavonoids in plasma and the fact that the presence of copper (II) bound to HSA in plasma is inherent to physiological conditions, we have undertaken the present pulse radiolysis study to look for possible effects of HSA-bound copper on oxidation reactions involving $^{\cdot}O_2^-$ in plasma. The reaction of $^{\cdot}O_2^-$ with the urate radical and the reduction of $^{\cdot}O_2^-$ by quercetin were chosen as model systems for these studies. We have also examined the effects of oxygen and HSA-bound copper on the urate radical and on the repair of this radical by HSA-bound quercetin in neutral buffered aqueous solutions.



Quercetin (QH_2)



Quercetin radical anion ($^{\cdot}Q^-$)

SCHEME 1

MATERIAL AND METHODS

All chemicals and solvents were of analytical grade and were used as received. Quercetin (QH_2) (Scheme 1), fatty acid-free HSA, and sodium urate (UH_2Na), were purchased from Sigma (St Louis, Mo, USA). The phosphate buffer (pH 7) was prepared in pure water obtained with a reverse osmosis system from Ser-A-Pure Co. The water exhibits a resistivity of >18 Mohms cm^{-1} and a total organic content of <10 ppb.

The optical absorption spectrophotometry of the air-saturated HSA solutions was carried out with a UVIKON 943 spectrophotometer. An SLM AMINCO-BOWMAN (series 2) was used for fluorescence measurements. Because of the poor solubility of QH_2 in pH 7 phosphate buffer, a stock solution of QH_2 (5 mM) was prepared in absolute ethanol, and then diluted to the desired concentration with the HSA solution before recording spectra.

Pulse Radiolysis

Pulse radiolysis measurements were carried out with the Notre Dame Radiation Laboratory 8 MeV linear accelerator, which provides 5 ns pulses of up to 30 Gy. In general, the doses used in this work were in the range 5–20 Gy. The principles of the detection system have been previously described.[18,19] A Corning O-52 optical filter, removing all wavelengths shorter than 350 nm, was placed in the analyzing light beam preceding the sample cell.

Radical concentrations calculated from transient absorption data are referenced to $^{\cdot}(SCN)_2^-$ dosimetry. The extinction coefficient for $^{\cdot}(SCN)_2^-$ is taken to be

$7580 \pm 60\,M^{-1}\,cm^{-1}$ at 472 nm, and the G value for $^{\cdot}OH$ in N_2O-saturated solution has been measured as 6.13 ± 0.09.[20] The G value is the number of radicals generated per 100 eV of absorbed energy. Such numbers may be recast as radical concentrations per unit radiation (e.g. a G value of 6.1 corresponds to a concentration of $0.63\,\mu M\,Gy^{-1}$ or $0.63\,\mu M\,J^{-1}\,kg$).

Solutions for pulse radiolysis were prepared in 10 mM pH 7, phosphate buffer. To avoid $^{\cdot}OH$ radical scavenging by ethanol (see above), QH_2 was directly dissolved at the desired concentration in the HSA solution. All solutions (generally 50 ml) also contained 0.1 M Br^-. They were saturated for 10 min with pure N_2O or O_2. Aliquots of a 10 mM stock aqueous solution of Cu^{2+} ions were added to the N_2O or O_2-saturated solutions of HSA or HSA + QH_2 immediately before kinetic measurements. Where necessary, differences in radical G value, arising from the dependence of spur scavenging on the Br^- concentration, were taken into account.[21] In addition, production of superoxide radical by oxygen scavenging of H· was included when calculating the G value for $^{\cdot}O_2^-$. Numerical integrations, carried out in analyses of rate data, were conducted using the *Scientist* software from Micromath Scientific Software.

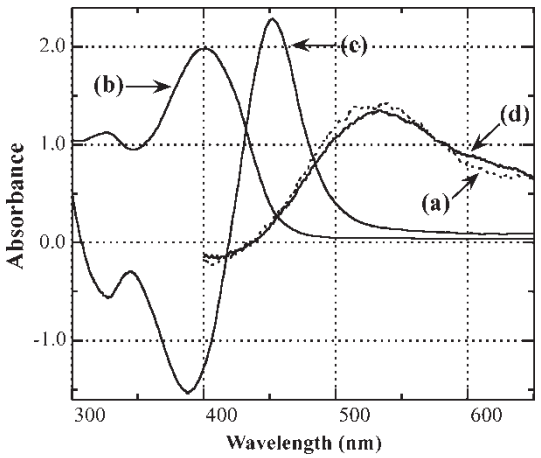


FIGURE 1 (a) Difference absorbance spectrum of a solution containing 100 μM HSA and 100 μM Cu^{2+} against that of a 100 μM HSA solution. The Cu^{2+} ions negligibly absorb in this wavelength range. (b) Absorbance spectrum of a solution containing 100 μM HSA and 100 μM QH_2. Quercetin is the only absorbing species in the 310–500 nm range. (c) Difference absorbance spectrum of a solution containing 100 μM HSA, 100 μM QH_2, and 100 μM Cu^{2+} against that of a solution containing 100 μM HSA and 100 μM QH_2. (d) Same as "a" but the solution also contained 1 mM urate. All spectra were recorded in pH 7, 10 mM phosphate buffer in a quartz cuvette with an optical path of 1 cm. Temperature was 20°C. Absorbances have been multiplied by 100 for "a" and "d" and by 10 for "c".

RESULTS AND DISCUSSION

Quercetin and Urate as Potential Ligands of HSA-bound Cu^{2+}

The binding of Cu^{2+} to HSA is accompanied by the appearance of absorption bands whose maxima depend on the $[Cu^{2+}]/[HSA]$ ratio.[22] Under physiological copper concentrations a 1:1 complex is formed predominantly with an equilibrium constant of $9 \times 10^6\,M^{-1}$.[8] This complex is characterized by an absorbance spectrum whose maximum is located at about 525 nm at neutral pH.[22] Figure 1a shows the optical absorption difference spectrum of a solution containing both copper (II) and HSA at equimolar concentration against that of a solution containing HSA alone. In plasma, QH_2 is carried by HSA to which it strongly binds in HSA domain IIA with an equilibrium constant of $2.6 \times 10^5\,M^{-1}$.[23] The absorbance of the QH_2-HSA complexes is shifted to the red as compared to that of QH_2 in buffer.[24] Figure 1b illustrates the red-shift observed in the optical absorbance spectrum of QH_2 upon complex formation in equimolar HSA and QH_2 solutions (100 μM).

Furthermore, flavonoids such as QH_2 have well known metal ion-chelating properties[25] and can chelate Cu^{2+} ions.[26] As a result, although QH_2 and copper ions separately bind to different sites in HSA, it must be determined whether the binding of QH_2 to HSA can perturb the HSA-Cu^{2+} complex formation. Figure 1c shows the difference spectrum of the QH_2-HSA complex in the presence and absence of equimolar Cu^{2+} ion concentration. Similar data were obtained with solutions whose concentrations of HSA, QH_2 and Cu^{2+} ions were varied from 0.5 μM to 200 μM (data not shown). These data, all demonstrate an absorbance shift of HSA-bound QH_2 to the red upon Cu^{2+} addition. This result is consistent with previous studies demonstrating the formation of Cu^{2+}-QH_2 complexes in methanolic solutions.[26] Our data strongly suggest that QH_2 can compete with the histidyl binding site (located in the N-terminal region of HSA) as a ligand of the Cu^{2+} ion. Incidentally, it may be noted that the absorbance difference spectrum reported in Fig. 1c is independent of the order of addition of QH_2 or Cu^{2+} to the HSA solution.

In order to ascertain whether a ternary complex involving HSA, QH_2, and Cu^{2+} is formed, the fluorescence spectra of (a) 2.5 μM HSA alone, (b) equimolar HSA and QH_2 (2.5 μM) and (c) equimolar HSA, QH_2 and Cu^{2+} (2.5 μM) were recorded in 10 mM, pH 7 phosphate buffer solutions. Because absorbances of these solutions were below 0.1, no inner filter effect on fluorescence intensities was observed (data not shown). Under excitation with 296 nm light, the HSA fluorescence at 344 nm is that of the single Trp residue (Trp 214) in domain IIA.[8] The addition of equimolar Cu^{2+} ions produced a 7% quenching of the HSA

fluorescence. Upon addition of QH_2 to the HSA solution, a strong quenching of the HSA fluorescence (82%) was observed. The efficiency of this quenching is consistent with an energy transfer from the first singlet excited state of the Trp residue to the corresponding state of QH_2 bound to the same domain[23] as the Trp residue. Addition of Cu^{2+} to the solution containing HSA and QH_2 produces a further 3% fluorescence quenching following the quenching by QH_2. These fluorescence data strongly suggest that copper (II) is displaced from its binding site located in domain I at the N-terminal to domain IIA where both Trp 214 and QH_2 lie.

As observed with the free Cu^{2+}-QH_2 complex in methanolic solutions, formation of a Cu^{2+}-QH_2 complex in air-saturated solutions of HSA induces a time-dependent degradation of bound QH_2. One hour after addition of Cu^{2+}, only 15% of the Cu^{2+}-QH_2 complex is still present in solution. The disappearance of the Cu^{2+}-QH_2 complex is accompanied by the appearance of a strong negative absorbance at 400 nm in the difference spectrum of the Cu^{2+}-QH_2 complex measured against that of the QH_2-HSA complex (data not shown). This band is shifted to the red by 10 nm as compared to that of the original spectrum shown in Fig. 1c. These irreversible absorbance changes have been attributed to the oxidation of QH_2 occurring at the 3-hydroxy group of ring C of QH_2 in methanolic solutions.[26]

It has been reported that uric acid or, more likely, the urate monoanion UH_2^- is a copper (II)-chelating agent at physiological pH.[27] Because no data were available, we have reinvestigated this possibility to obtain more quantitative results. However, using either second derivative absorption or fluorescence spectroscopies (data not shown), we were unable to find any evidence for copper (II)-urate complex formation. In the light of these conflicting observations, we therefore, verified that a 10-fold excess of urate cannot displace copper (II) from HSA under our experimental conditions (see Fig. 1d).

Effect of Cu^{2+} on Semi-oxidized Urate in the Presence and Absence of O_2

Pulse radiolysis of an N_2O-saturated buffered aqueous solution containing 0.1 M Br^- generates $^{\cdot}Br_2^-$ radical-anions by the sequence of reactions:

$$H_2O \rightarrow e_{aq}^-, H^{\cdot}, {}^{\cdot}OH, H_2O_2$$

$$e_{aq}^- + N_2O + H_2O \rightarrow {}^{\cdot}OH + N_2 + OH^- \qquad (I)$$

$${}^{\cdot}OH + 2Br^- \rightarrow {}^{\cdot}Br_2^- + OH^- \qquad (II)$$

Under these experimental conditions, $^{\cdot}Br_2^-$ radical anion is produced with a radiolytic yield of 6.2 ($0.64\,\mu M\,Gy^{-1}$). In the presence of 1–2 mM UH_2^-, the $^{\cdot}Br_2^-$ radical reacts stoichiometrically with UH_2^-.

$${}^{\cdot}Br_2^- + UH_2^- \rightarrow 2Br^- + {}^{\cdot}UH^- + H^+ \qquad (III)$$

The $^{\cdot}UH^-$ radical transient exhibits a maximum absorbance at 325 nm ($\varepsilon_{max} = 8,200\,M^{-1}\,cm^{-1}$).[12] The rate constant for this reaction is $8.3 \times 10^8\,M^{-1}\,s^{-1}$.[10,12] In 10 mM pH 7 buffer, $^{\cdot}UH^-$ decays via the bimolecular reaction:

$${}^{\cdot}UH^- + {}^{\cdot}UH^- \rightarrow \text{products} \qquad (IV)$$

with a rate constant $2k_{IV} = 3.4 \times 10^8\,M^{-1}\,s^{-1}$.[16] In the presence of 200 μM HSA, the high concentration of urate: i.e. a 10-fold excess as compared to [HSA], ensures that $^{\cdot}Br_2^-$ reacts predominantly with UH_2^- in bulk solution (or possibly UH_2^- bound to HSA) rather than with HSA itself. The rate constant for the one-electron oxidation of HSA by $^{\cdot}Br_2^-$ is $2 \times 10^9\,M^{-1}\,s^{-1}$[11] (Fig. 2A). The reaction rate constant for the $^{\cdot}UH^-$ decay is found to be $2k_{IV} = 5.6 \times 10^8\,M^{-1}\,s^{-1}$ in the HSA solution. This value is in good agreement with that previously measured with the $^{\cdot}UH^-$ decay in buffer. The finding supports the assumption that $^{\cdot}UH^-$ radical is predominantly formed in buffer alone. Decay of the $^{\cdot}UH^-$ radical at 400 nm was also measured in N_2O-saturated solutions containing concentrations of Cu^{2+} sufficient to form a 1:1 complex with HSA. The kinetics reported in Fig. 2A demonstrate that the presence of the Cu^{2+}-HSA complex has little effect on $^{\cdot}UH^-$ decay.

We have previously shown that the $^{\cdot}UH^-$ radical reacts with the superoxide radical-anion, $^{\cdot}O_2^-$ according to the reaction:

$${}^{\cdot}UH^- + {}^{\cdot}O_2^- \rightarrow \text{products} \qquad (V)$$

with a rate constant $k_V = 8.3 \times 10^8\,M^{-1}\,s^{-1}$[12] although $^{\cdot}UH^-$ does not react with oxygen itself.[10] It is well known that $^{\cdot}O_2^-$ can readily react with free Cu^{2+} ions and many histidyl-Cu^{2+} complexes at almost diffusion-controlled rates.[28] It may thus be anticipated that HSA-bound Cu^{2+} may deactivate $^{\cdot}O_2^-$ in competition with reaction (V). To investigate such possible competition between the HSA-Cu^{2+} complex and $^{\cdot}UH^-$ for the deactivation of $^{\cdot}O_2^-$, the time dependent behavior of the $^{\cdot}UH^-$ radical was studied by pulse radiolysis in O_2-saturated buffered solutions containing 50–200 μM HSA, 1–2 mM urate, and 0.1 M Br^-. Under these experimental conditions, $^{\cdot}UH^-$ is produced via reactions (II) and (III) with a radiolytic yield of 2.7. The other two radicals generated by radiolysis of water, $H^{\cdot}$ and hydrated electrons (e_{aq}^-), react with oxygen leading to $^{\cdot}O_2^-$ formation (radiolytic yield 3.3). The decay kinetics in Fig. 2B demonstrate that the HSA-copper (II) complex has only a limited effect on reaction (V). Since $^{\cdot}UH^-$

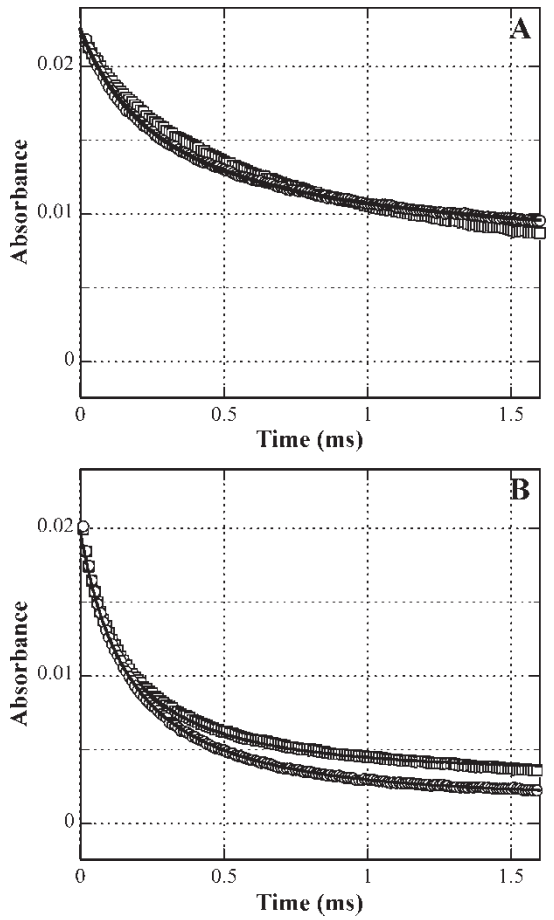


FIGURE 2 (A) (○) Decay of the transient absorption of the $^{\cdot}UH^{-}$ radical measured at 400 nm after pulse radiolysis of an N_2O-saturated 10 mM pH 7 buffered solution containing 100 mM KBr, 200 μM HSA and 2 mM urate. Temperature: 18°C. Dose was 9.5 Gy. (□) Same conditions as in (○) but the solution also contained 200 μM Cu^{2+}. The solid line shows the fit of transient absorbance changes for the copper-free solutions assuming second order kinetics (see text). (B) The solutions described in Fig. 2A have been saturated with O_2 instead of N_2O. For the sake of easy comparison between Figs. 2A and B, transients shown in Fig. 2B have been obtained with a dose of 19 Gy which approximately leads to the same $^{\cdot}UH^{-}$ radical concentration as in Fig. 2A because $G(^{\cdot}Br_2^{-})$ are 6.2 and 2.7 in N_2O and O_2-saturated solutions, respectively. (○) no Cu^{2+}, (□) 200 μM Cu^{2+}. The solid lines show the fits of transient absorbance changes according to Eq. 1 (⊖) and Eq. 2 (⊟).

and $^{\cdot}O_2^{-}$ are formed in the bulk, an estimate of the rate constant (k_{VI}) for the pseudo-first order reaction:

$$Cu^{2+} - HSA + {}^{\cdot}O_2^{-} \rightarrow \text{products} \qquad \text{(VI)}$$

can be obtained from the rate equations:

$$-d[{}^{\cdot}UH^{-}]/dt = 2k_{IV}[{}^{\cdot}UH^{-}]^2 + k_V[{}^{\cdot}O_2^{-}][{}^{\cdot}UH^{-}] \quad (1)$$

and

$$-d[{}^{\cdot}O_2^{-}]/dt = 2k[{}^{\cdot}O_2^{-}]^2 + k_V[{}^{\cdot}O_2^{-}][{}^{\cdot}UH^{-}] + k_{VI}[{}^{\cdot}O_2^{-}][Cu^{2+} - HSA] \qquad (2)$$

At neutral pH, the dismutation reaction, $^{\cdot}O_2^{-} + {}^{\cdot}O_2^{-} \rightarrow H_2O_2$, occurs with a much slower rate constant ($k < 10^6\,M^{-1}\,s^{-1}$[28]) than that for reaction (V) (see above), and thus the dismutation reaction was neglected in our analysis. With this kinetic model, an excellent fit of the $^{\cdot}UH^{-}$ radical decay is obtained (see Fig. 2B) with values of $2k_{IV} = 5.8 \times 10^8\,M^{-1}\,s^{-1}$, close to that determined above in the absence of oxygen, and $k_V = 5.7 \times 10^8\,M^{-1}\,s^{-1}$, yielding a value of $k_{VI} = 6.1 \times 10^6\,M^{-1}\,s^{-1}$. The relatively small magnitude of this value indicates that, under physiological concentration conditions, the HSA-bound copper fraction must be considered as a poor reactant for the superoxide radical-anion. Furthermore, the reactivity of the copper (II)-HSA complex is more than three order of magnitude lower than that of the plasma Cu–Zn superoxide dismutase (rate constant of reaction with $^{\cdot}O_2^{-} \sim 2.3 \times 10^9\,M^{-1}\,s^{-1}$[28]). As a consequence it is unlikely that it can compete with the enzyme for the deactivation of $^{\cdot}O_2^{-}$ in plasma.

Effect of Copper and Oxygen on the Repair of Semi-oxidized Urate by HSA-bound Quercetin

It has previously been established that in N_2O-saturated solutions, HSA-bound QH_2 can repair the $^{\cdot}UH^{-}$ radical produced by $^{\cdot}Br_2^{-}$ via the electron transfer reaction:

$$^{\cdot}UH^{-} + QH_2 \rightarrow {}^{\cdot}Q^{-} + UH_2^{-} + H^{+} \qquad \text{(VII)}$$

The rate constant was found to be $k_{VII} = 2 \times 10^6\,M^{-1}\,s^{-1}$. We have previously established[11] that the transient absorbance spectrum obtained by oxidation of HSA- bound QH_2 has an absorbance maximum at 570 nm corresponding to that of the quercetin monoanion $^{\cdot}Q^{-}$[29] shown on Scheme 1. Figure 3A shows the growth of the transient absorbance of $^{\cdot}Q^{-}$ recorded at 620 nm after pulse radiolysis of an N_2O-saturated buffered solution containing 2 mM UH_2^{-}, equimolar (200 μM) HSA and QH_2, and 10 mM Br^{-}. This wavelength was chosen because it corresponds to the wavelength of the isosbestic point between the $^{\cdot}Q^{-}$ spectrum and that resulting from the subsequent transformation of $^{\cdot}Q^{-}$ radicals. The transient absorbances measured at 620 nm are, therefore, independent of the identity of the QH_2 species absorbing at this wavelength.[11] Based on a molar absorbance of $3{,}100\,M^{-1}\,cm^{-1}$, the G value for the repair of $^{\cdot}UH^{-}$ by electron transfer from QH_2 measured 1.5 ms after the radiolytic pulse was found to be 0.05. Incidentally, although rather low, this G value is almost 10 times larger than that reported in ref. 11 over a similar time interval, because the HSA solutions in the current work contained 200 μM QH_2 as opposed to 25 μM QH_2 in the previous study. We have attributed this low repair

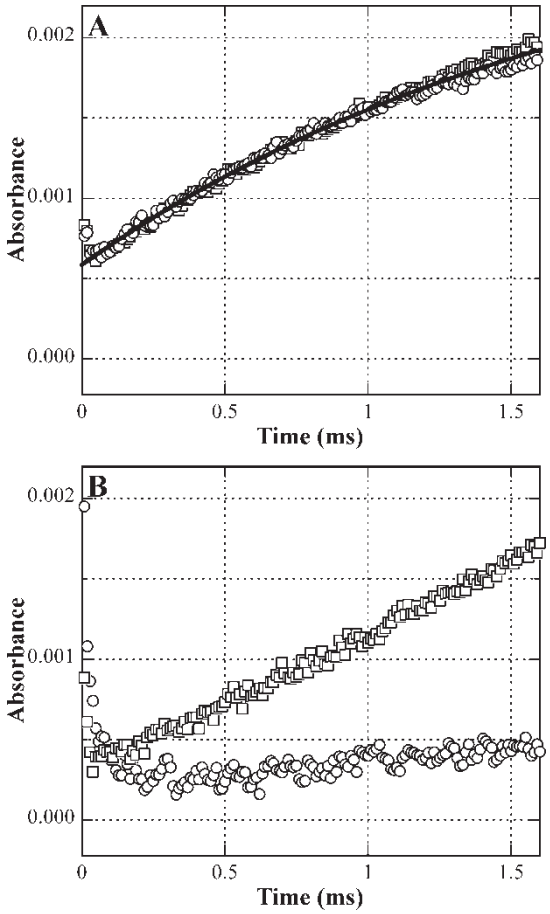


FIGURE 3 (A) Growth of the transient absorption of the $^{\cdot}Q^{-}$ radical measured at 620 nm after pulse radiolysis of an N_2O-saturated 10 mM pH 7 buffered solution containing 100 mM KBr, 200 μM HSA, 200 μM QH_2, and 2 mM urate. The excellent experimental reproducibility is illustrated by this figure (note the low absorbances) since this growth is the average of three independent determinations each of them resulting from signal averaging of 20 kinetic traces using the same radiolytic dose. Temperature: 18°C. Dose was 20 Gy. (□) Same conditions as in (○) but the solution also contained 200 μM Cu^{2+}. The solid line shows the fit of transient absorbance changes assuming pseudo first order kinetics (see text). Note that the fast decaying initial transient absorbance is due to the $^{\cdot}Br_2^{-}$ radical-anions. (B) Growth of the transient absorption of the $^{\cdot}Q^{-}$ radical measured at 620 nm after pulse radiolysis of an O_2-saturated 10 mM pH 7 buffered solution containing 100 mM KBr, 200 μM HSA, 200 μM QH_2 and 2 mM urate. Temperature: 18°C. Dose was 19.5 Gy. (□) Same conditions as in (○) but the solution also contained 200 μM Cu^{2+}. Here, e_{aq}^{-} and $^{\cdot}Br_2^{-}$ contribute to the fast initial decay.

efficiency to a reduced accessibility of the urate radical to HSA-bound QH_2.[11] The kinetics shown in Fig. 3A demonstrate that the growth rate of the $^{\cdot}Q^{-}$ radicals is independent of the presence or absence of HSA-bound Cu^{2+}. Therefore, the addition of Cu^{2+} changes neither the repair efficiency nor the rate of reaction (VII). A k_{VII} of $2.7 \times 10^6\,M^{-1}\,s^{-1}$ can be estimated from the growth kinetics assuming pseudo-first order kinetics for reaction (VII) which yields a rate constant of $540\,s^{-1}$. This value is fully consistent with that previously reported.[11] Since under these conditions, Cu^{2+} ions are chelated by HSA-bound QH_2, the value suggests that the copper (II)-QH_2 complex can still repair $^{\cdot}UH^{-}$.

In O_2-saturated solutions, the repair of $^{\cdot}UH^{-}$ by QH_2, (reaction VII) is strongly inhibited (see Fig. 3B) due to the consumption of $^{\cdot}UH^{-}$ by the fast reaction (V) which competes effectively with the electron transfer process (VII). However, addition of equimolar Cu^{2+} ions restores the formation of $^{\cdot}Q^{-}$ possibly because the copper (II)-QH_2 complex can effectively react with the $^{\cdot}O_2^{-}$ radicals formed in the bulk thus favoring reaction (VII). Alternatively, it may be possible that the copper (II)-QH_2 complex can directly produce $^{\cdot}Q^{-}$ radicals by catalyzing the reduction of the $^{\cdot}O_2^{-}$ radicals by QH_2. This reduction has been shown to occur in homogenous[30] or micellar[11] solutions. Experimental limitations, e.g. low yield of the electron transfer and transient absorbance overlap, preclude favoring one of these two hypotheses.

CONCLUSION

The main findings of this study are:

Quercetin can displace HSA-bound copper (II) from its binding site. Such a copper relocalization induced by quercetin, one of the most common flavonoids, may possibly affect copper delivery to tissues.

In the presence of oxygen, the repair of the urate radical by quercetin involves copper (II)-bound quercetin under concentration conditions implying formation of a 1:1:1 Cu^{2+}-QH_2-HSA complex. In the circulatory system, the urate radicals may be produced by $^{\cdot}OH$ radicals[9] formed by Fenton-like reactions. Such reactions are involved, for instance, in the oxidative burst accompanying the leucocyte functions which generate $^{\cdot}O_2^{-}$ radicals as well. However, under physiological conditions, it should be noted that due to the large HSA and urate concentrations in contrast to the relatively low copper (II) and QH_2 concentrations, there will be an excess of complex-free HSA molecules. All the complexes, described here, are likely to co-exist in solution with equilibria driven by their stability constants and the relative concentrations of molecules involved. Furthermore, molecular interactions, similar to those described here for HSA, may possibly be found with other circulating proteins. In this regard, it would be interesting to study whether such ternary complexes may be involved as well in the contrasting pro and anti-oxidant effects of urate in the copper-induced LDL oxidation[31,32] and in synergistic effects of urate and flavonoids affecting the inhibition of the Cu^{2+}-induced plasma lipid peroxidation previously reported by us.[17]

Acknowledgements

We wish to thank M. Bazin for useful discussions. PF, PM and RS are recipients of an "INSERM—Instituto de Cooperação Cientifica e Tecnológica Internacional" grant. Notre Dame Radiation Laboratory is supported by the Office of Basic Energy Sciences of the US Department of Energy. This is document No 4433 from the Notre Dame Radiation Laboratory.

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