Vol. 195, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1993 Pages 113-119

ROLE OF IRON ION CHELATION BY QUINONES IN THEIR REDUCTION, OH-RADICAL GENERATION AND LIPID PEROXIDATION

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Received July 6, 1993

To study the role of the complex of quinones with iron ions in the processes of quinone reduction and OH-radical generation in the presence of ascorbate (AH) and glutathione (GSH) the quinone-chelators have been used: 2-phenyl-4-butylaminonaphtho[2,3-h]quinolindione-7,12 (Qc) and adriamycin (Adr). 2-Phenyl-5-nitronaphtho[2,3-g]indodione-6,11 (Qn), 2-(3-hydroxypropyl)anthraquinone (AQOP) and 2-dimethylamino-3-chlor-1,4-naphthoquinone (DCNQ) were chosen as quinones that do not chelate iron ions. It was found that, unlike Adr and nonchelating quinones Qn, AQOP, and DCNQ, addition of Qc to AH and GSH leads to semiquinone EPR spectrum formation and OH-radical generation via the complex of Qc with iron ions. It was demonstrated that all these quinones can be reduced by AH. However, reduction constant of Qc-Fe(3+) by the AH was 98±9 $M^{-1}c^{-1}$, while DCNQ reduction constant was only 0.042±0.005 M^{-1} c^{-1}. It was found that in the presence of GSH only complexes of quinones Qc and Adr with iron ions are reduced. It is concluded that the capability of Qc to reduce and to generate OH-radicals is related to intramolecular electron transfer by the reaction: $Fe(2+)\text{-}Qc \Leftrightarrow Fe(3+)\text{-}Qc^{\bullet}$. The capability of Qc to increase generation of oxygen radicals and to inhibit lipid peroxidation may be interesting for designing quinone-containing antibiotics.

The quinone-containing compounds serve as the basis for most of available antitumor antibiotics (1). It is assumed that the ability of quinones to be reduced in intact cells and to generate OH-radicals is a major precondition for manifestation of their antitumor activity (2,3).

As has been shown by a number of authors, the antibiotics rubomycin and adriamycin can form complexes with Fe (3+) that more actively generate oxygen radicals and damage DNA in the enzymatic system and with GSH (4). It is also known that quinones can more efficiently suppress the development of tumor cell cultures in the presence of AH (5). Therefore, an assumption has been made on the important role of the iron-quinone complex in the reduction of quinones and formation of OH-radicals for the increasing of the drug antitumor action.

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0006-291X/93 $4.00

Earlier, OH-radical generation by chelating quinones has been studied in the system with NADPH cytochrome P-450 reductase (6). It was shown that Qc is more effective in OH-radical generation than Adr and quinones-nonchelators.

In this paper we have investigated the role of iron ion chelation by quinones in their reduction and OH-radical generation in the presence of glutathione and ascorbate as well as the influence of chelating quinones on the lipid peroxidation.

MATERIALS AND METHODS

Qc, Qn, AQOP, DCNQ were kindly supplied by Piskunov A.V. (Institute of Chemical Kinetics & Combustion). These quinones were synthesized as described in (6,7,8). Adriamycin (Adr), Desferrioxamine (Df) and Trizma base was obtained from Sigma (USA), NADPH was supplied by Reanal (Hungary). 1mM aqueous solution of $FeCl_3$ (Serva, FRG) was used for 1 hour after preparation. Spin trap 2-dimethylpyrroline-N-oxide (DMPO) (Sigma, USA) was purified as described in (9) In EPR experiments the sample contained either AH 2mM or GSH 10mM, 15% dimethylsulfoxide (DMSO), 0.1 mM quinones, 0.1 M DMPO in 50mM tris-HCl buffer, 0.1M KCl, pH=7.40

OH-radical formation was followed by the ESR spectrum of spin adduct DMPO-CH_3 forming under the action of OH-radicals on DMSO (10). The ESR measurements were performed using an ER-200D-SRC (Bruker) spectrometer in a sealed quartz ampoule with volume 0.2 ml. The conditions for recording ESR spectra are following: field center is 3474 G, microwave power is 20 mW, modulation amplitude is 1G, gain is $5 \cdot 10^5$.

A microsomal fraction of rat liver was isolated as described in (11). Protein content was determined by the Lowry method (12). Lipid peroxidation was estimated by thiobarbituric acid reactive substances (TBARS) formation (13).

Oxygen consumpsion was determined in the samples containing 25% dimethylsulfoxide, 5μM $FeCl_3$ and 2.5 mM AH, or 50 μM $FeCl_3$ and 7.5 mM GSH, in the 50 mM tris-HCl, pH=7.4. It was followed by Clark-type electrod using oxygen monitor model 5221 (Soyuzpribor, Russia). Oxygen uptake was expressed as the initial rate (μM/s) of kinetics of oxygen reduction or as ratio of oxygen uptake with quinones to control level without one in %.

RESULTS AND DISCUSSION

Semiquinone formation and OH-radical generation in the presence with ascorbate and glutathione

The ESR semiquinone signal was observed in the solution of 0.1 mM Qc after addition of AH (Fig.1a) and GSH (Fig.2a). Addition of 2 mM Df to samples with AH decreased the ESR signal amplitude of semiquinone in 4 times (Fig.1b). No ESR spectrum of Qc semiquinone has been recorded in the presence of 10 mM Df and AH (Fig.1c) (only ascorbate anion radical dublet ESR spectrum was observed) or GSH (Fig.2c). But after the addition of 0.1 mM $FeCl_3$ to sample with GSH, the ESR signal amplitude increases in two times (Fig.2b).

When AH or GSH is added to Adr, AQOP, DCNQ, Qc, the ESR spectra of semiquinones are not observed.

OH-radical formation has been studied by the measurement of DMPO-CH_3 spin adduct in solution of quinones with AH or GSH. After addition of Qc to AH solution, the amount of spin adduct has increased in 3-5 times. The addition of Adr, Qn, DCNQ, AQOR has not stimu-

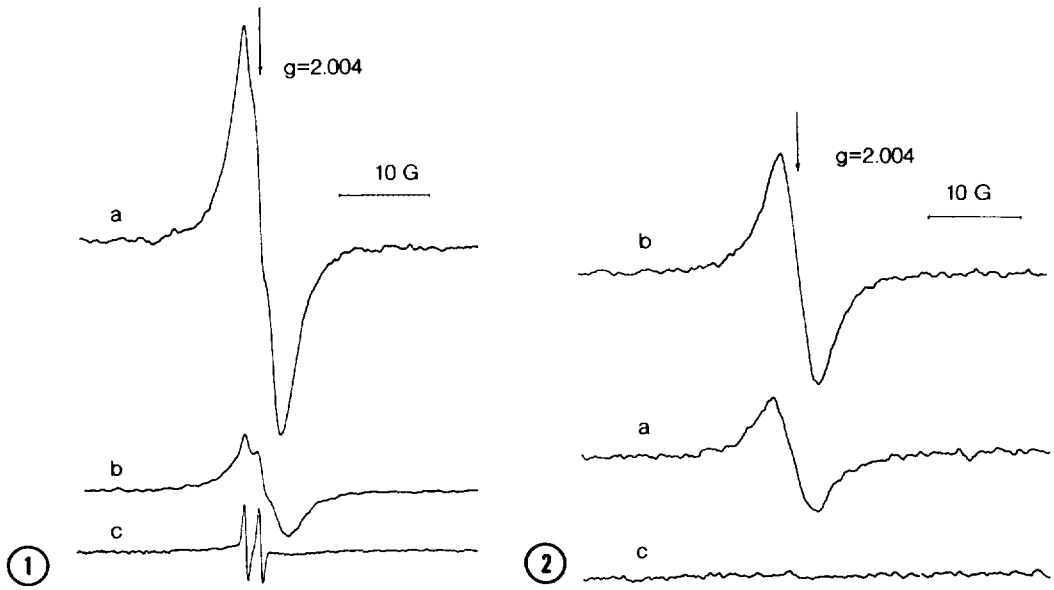

Fig.1. EPR spectrum of the solution of 0.1 mM Qc in the presence of 1 mM ascorbate (a); after addition of 2 mM Desferrioxamine (b); after addition of 10 mM Desferrioxamine (c).

Fig.2. EPR spectrum of the solution of 0.1 mM Qc in the presence of 4 mM glutathione (a); after addition of 0.1 mM $FeCl_3$ (b); after addition of 10 mM Desferrioxamine (c).

lated OH-radical formation. In the presence of GSH addition of Qc lead to DMPO-thyil radical spin adduct formation (data not shown). But the other quinones fail to do it.

Fe(3+) ions are known to be reduced by AH and GSH to Fe(2+) ions. The reaction can be stopped if Fe(3+) ions are bound by Df (14). Therefore, the fact that the chelating quinone Qc does not reduce in the presence of 10mM Df testifies that the reduction is possible upon interaction between either the chelating quinone and Fe(2+) or the Qc-Fe(3+) complex and reductants. In turn stimulation of Qc semiquinone formation in the presence of GSH by the addition of $FeCl_3$ (Fig.2a, 2b) also points out the importance of iron ions in Qc reduction. Since neither Adr nor Qn, nor DCNQ nor AQOP cannot reduce to semiquinones by AH or GSH, the reactions of Qc reduction can proceed in the following way:

1. $Fe(3+) + DH \Rightarrow Fe(2+) + D^{\cdot} + H^{+}$
2. $Fe(2+) + Qc \Rightarrow Qc\text{-}Fe(2+)$
3. $Qc\text{-}Fe(2+) \Leftrightarrow Fe(3+)\text{-}Qc^{\cdot}$ DH = AH or GSH

The existence of reaction 2 and 3 is also confirmed by the fact that only in the case of Qc the addition of $FeSO_4$ causes the appearance of semiquinone ESR spectrum.

The fact of semiquinone formation in the presence of 2 mM Df when Fe(3+) ions are bound to either Df or Qc (Fig.1b), suposes another mechanism of Qc-Fe(2+) formation:

4. $Qc + Fe(3+) \Leftrightarrow Qc\text{-}Fe(3+)$
5. $Qc\text{-}Fe(3+) + DH \Rightarrow Qc\text{-}Fe(2+) + D^{\cdot} + H^{+}$

Table 1. Oxygen uptake (μM/c) in the samples containing ascorbate and Qc-Fe(3+) or DCNQ

AH					
2.5 mM			5 mM		
Qc-Fe(3+), μM			DCNQ, mM		
1	3	5	1	2	3
0.25	0.75	1.2	0.2	0.43	0.64

Oxygen consumption in the presence of the ascorbate

It was known that ascorbate was oxidized by Fe(3+) forming Fe(2+) which oxidized by oxygen. So without quinones oxygen consumption occur due to iron catalized ascorbate oxidation and then Fe(2+) oxidation by O_2. It is a control level of oxygen uptake.

It was found that quinones studied stimulate oxygen uptake (tables 1 and 2). Oxygen consumption was linearly depends on concentration of quinones (Qn, DCNQ, AQOP) or Fe(3+)-Qc and ascorbate (table 1). Oxygen uptake in these samples may be explaned by the next scheme. On the first step semiquinone is formed (reaction 6). Then semiquinone is oxidized by oxygen (reaction 7). Since the reaction 7, 8, 9 and 10 are very fast we supose that the rate determining stage of oxygen uptake is the quinone reduction. If so, the rate of oxygen uptake would be equal to the rate of quinone reduction. To verify this suggestion we have calculated the constant of reaction 6.

6. $DCNQ + AH^- \Rightarrow DCNQ^- + A^{\cdot} + H^+$
7. $DCNQ^{\cdot} + O_2 \Rightarrow DCNQ + O_2^{\cdot}$
8. $O_2^{\cdot} + H_2O \Rightarrow HO_2^{\cdot} + OH^-$
9. $HO_2^{\cdot} + AH^- \Rightarrow H_2O_2 + AH^{\cdot}$
10. $HO_2^{\cdot} + AH^{\cdot} \Rightarrow H_2O_2 + A$

$V_6 = k_6 [DCNQ] [AH]$, $V_6 = Vo_2$, $k_6 = Vo_2 / [DCNQ] [AH]$,

where V_6 and k_6 are the rate and constant of reaction 6, and Vo_2 is the rate of oxygen uptake, AH and A are ascorbic acid in reduced and oxidized form, consequently.

The bimolecular rate contant of reaction 6 was obtained, $k_6 = 0.042 \pm 0.005$ $M^{-1}c^{-1}$. This value is closed to the one for the orthobenzoquinones (15). The very low value of k_6 compared to k_7, k_8, k_9 and k_{10} (for Adr $k_7 = 4.4 \cdot 10^7$ $M^{-1}c^{-1}$ (16), $k_8 \cong 10^{10}$ $M^{-1}c^{-1}$, $k_9 = 5 \cdot 10^4$ $M^{-1}c^{-1}$ and $k_{10} = 2.6 \cdot 10^8$ $M^{-1}c^{-1}$ (17)) supports the rate determining stage 6 in oxygen consumption.

Since the DCNQ has the highest oxidizing potential among quinones studied we proposed that oxygen consumption quantitavly follows to reduction of Qn and AQOP.

As we shown above, Qc in the complex with Fe(3+) can be reduced by ascorbate to semiquinone. In support of the participation of Qc-Fe(3+) in the oxidation of ascorbate we have not found the dependance of oxygen uptake both on Fe(3+) at concentration higher than concen-

Table 2. Oxygen uptake in the samples containing 2.5 mM ascorbate, 5μM $FeCl_3$, Df and quinones is shown in the tables. Oxygen uptake in the sample without quinone and Df is 100%.

Quinone / Df	Qc 5μM	Qn 0.2mM	Adr 0.5mM	AQOP 0.5mM	DCNQ 0.5mM
____	1635±85%	209±17%	409±31%	262±28%	566±34%
150μM	625±45%	196±17%	270±22%	____	476±56%

tration of Qc, and on Qc concentration in the samples without Fe(3+) added. Therefore oxygen uptake may occur by reactions 5, 3, 11 and 8, 9, 10 where reaction 5 is rate determining stage.

11. $^{\bullet}$Qc-Fe(3+) + O_2 ⇒ Qc-Fe(3+) + $O_2^{\bullet-}$

$V_5=k_5$ [Qc-Fe(3+)] [AH], $V_5=Vo_2$, $k_5=Vo_2/$[Qc-Fe(3+)] [AH] , where V_5 and k_5 are the rate and rate constant of reaction 5 consequently.

Using oxygen uptake data (table 1) reduction constant of Qc-Fe(3+) by ascorbate 98±9 M^{-1} c^{-1} was obtained. It is much lower than the rate constants of semiquinones oxidation by oxygen. Thus the rate of oxygen uptake is equal to rate of Qc-Fe(3+) reduction and value obtained is correct. It is necessary to note that quinone chelating iron ions had reduction constant in 2300 times higher than quinone nonchelator DCNQ.

The table 2 presents the data of oxygen uptake in the presence of quinones studied. It demonstrates that all quinones are reduced by AH. But addition of Df decrease stimulation of oxygen uptake only in the case of Qc and Adr chelating iron ions. It support the oxidation of ascorbate due to interaction with complexes Qc-Fe(3+) and Adr-Fe(3+). These complexes oxidized AH more effectivly than quinone not chelating iron ions. According to polarographic data (6), a higher efficiency of Qc reduction and OH-radical generation is not due to the higher oxidative characteristics of Qc (both free and in a complex) compared to other quinones.

Oxygen consumption in the presence of the glutathione

Table 4 show the oxygen uptake data in presence of GSH. It was found that quinones Qn, AQOP, DCNQ which do not chelate iron ions do not stimulate oxygen uptake. Quinones chelating iron ions Qc and Adr stimulate oxygen consumption at lower level compared to ascorbate system (table 3) and Df inhibited it. Moreover Df inhibited stimulation of oxygen uptake by Adr to the control without quinones. In the case of Qc addition of the same quantity of Df does not lead to total inhibition of oxygen uptake stimulation but only decreased the this rate in six times.

The absence of oxygen uptake stimulation by quinones nonchelators AQOP, DCNQ, Qn supported the idea that quinones are not reduced by glutathione directly. However quinones in the complex with iron ions Qc-Fe(3+) and Adr-Fe(3+) were able to stimulate oxygen consumption. In last case Qc forms semiquinone while Adr does not. We suppose that Qc was

Table 3. Oxygen uptake in the samples containing 7.5 mM glutathione, 50μM $FeCl_3$, Df and quinones is shown in the tables. Oxygen uptake in the sample without quinones and Df is 100%.

Quinone / Df	Qc 115μM	Qn 115μM	Adr 430μM	AQOP 430μM	DCNQ 1.5 mM
___	535±36%	103±8%	265±25%	102±8%	111±8%
130μM	178±16%	30±5%	33±5%	___	___

reduced according to reactions 4, 5, 3 where DH is glutathione. While Adr is likely to stimulate oxygen uptake through reactions 12 and 13.

12. Adr-Fe(3+) + GSH ⇒ Adr-Fe(2+) + GS• + H^+
13. Adr-Fe(2+) + O_2 ⇒ Adr-Fe(3+) + $O_2^{\bullet}$

The influence of quinones, chelating iron ions, on lipid peroxidation

Iron ions are known to be necessary for development of LP (18). Moreover, it is assumed that the side cardiotoxic action of Adr is connected with the activation of LP by the complex of Adr with iron ions (2). We have compared the influence of chelating quinones Qc and Adr on LP in a microsomal system of rat liver (Table 1). Adr increases TBARS contents and this process has been inhibited by Df. On the contrary, Qc not only fails to stimulate the TBARS formation but even decreases it compared to the control level without quinones. The Adr-Fe(3+) complex has stimulated TBARS formation to a larger degree than $FeCl_3$.

The addition of Qc, like Df, inhibited LP. The content of TBARS in the sample with Qc-Fe(3+) was less than after addition of $FeCl_3$ but higher than the control without iron ions. This is, probably connected with existance of free iron ions which increase TBARS formation compare to Qc. The role of semiquinone Qc in LP is not clear yet because LP inhibition after addition of Qc can be determined by two processes: 1) iron ions are chelated in a complex with Qc where they can not stimulate LP; 2) semiquinone Qc having scavenging properties breaks the chain of LP reactions.

Table 4. The influence of quinones chelating iron ions on lipid peroxidation

Quinone / Df	____	Adr	Qc	$FeCl_3$	Fe-Adr	Fe-Qc
____	1.2±0.2	2.1±0.2	0.4±0.2	6.1±0.4	8.0±0.5	3.5±0.3
50μM	-0.4±0.2	0±0.2	-0.7±0.2	____	____	____

TBARS contents (μM/mg protein/ml) in microsomal system (2 mg protein/ml) after 1 hour of incubation in the presence of 2 mM HADPH and components (50 μM) are shown in the table.

CONCLUSION

Therefore it was demonstrated that all quinone studied can be reduced by ascorbate and stimulate oxygen consumption. But Qc and Adr do it more effectively in the complexes with iron. Moreover reduction constant of Qc-Fe(3+) by the ascorbate was 98 ± 9 $M^{-1}c^{-1}$, while DCNQ reduction constant was only 0.042 ± 0.005 $M^{-1}c^{-1}$.

It was found that in the presence of glutathione only quinones chelating iron ions Qc and Adr in the complexes with Fe(3) are capable to oxidize GSH and stimulate oxygen consumption. But, unlike Adr, Qc was reduced to semiquinone.

Since Qc suppresses LP it is assumed that the quinone-containing antibiotics of the Qc type could be less cardiotoxic.

ACKNOWLEDGMENT

The authors are thankful to Dr. V. Khramtsov (Institute of Chemical Kinetics & Combustion) for fruitful discussions and his comments on the manuscript.

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