Semiquinone anion radicals formed by the reaction of quinones with glutathione or amino acids

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Received 14 April 1986

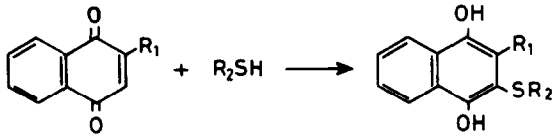
Using ESR spectroscopy, we show that benzoquinone, 1,4-naphthoquinone and 5-hydroxy-1,4-naphthoquinone react readily with thiol containing compounds, such as glutathione, to form their respective semiquinone anion radicals. These quinones react similarly, but less readily, with the amino group of amino acids. The therapeutic or toxicological significance of the formation of semiquinone anion radicals from the reaction of quinones with nucleophiles, such as thiols and amines, remains to be assessed.

Quinone *Thiol* *Amino acid* *ESR* *Semiquinone anion radical*

1. INTRODUCTION

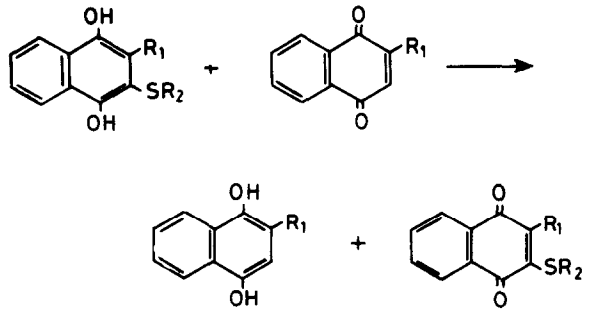
Quinones are found widely distributed in nature [1] and play a vital role in certain cellular functions. Some quinones, for example adriamycin [2], possess antitumour activity and are important therapeutically.

The addition and substitution reactions of quinones with nucleophiles, particularly thiol and amino groups, have been extensively documented [3–10] and comprehensively reviewed by Finley [11]. The majority of the reactions of quinones involve nucleophilic Michael addition [11] resulting in the formation of the hydroquinone conjugate (eqn 1).



(1)

The conjugate may then, depending on its structure, be oxidised either by unreacted parent quinone (eqn 2), oxygen, or chemical oxidising agents to the substituted quinone [11]. If the oxidation is performed by the parent quinone then the corresponding hydroquinone is formed.



(2)

Glutathione (GSH) is the major non-protein sulphydryl found in the cell, and plays a key role in cellular defence against chemical and radiation

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00145793/86/$3.50

induced damage [12]. Thus, much attention has focussed on the reaction of quinones with GSH. During the reaction of menadione (2-methyl-1,4-naphthoquinone) with GSH, Nickerson et al. [3] have shown the formation of both the hydroquinone (menadiol) and the oxidised conjugate. Ross et al. [13] have extended this work and shown the formation of oxidised glutathione (GSSG) and the superoxide anion radical (O_2^-) during the reaction.

In this study we demonstrate, using the technique of ESR, that a number of structurally simple quinones react with thiols and amines to yield their corresponding semiquinone anion radicals.

2. MATERIALS AND METHODS

p-Benzoquinone (BQ) and dimethyl sulphoxide (DMSO) were obtained from British Drug House, Poole, Dorset; 1,4-naphthoquinone (1,4-NQ) from Fluka, Switzerland and 5-hydroxy-1,4-naphthoquinone (5-OH-1,4-NQ) from Aldrich, Gillingham, Dorset. GSSG was obtained from Sigma, Poole and GSH from Boehringer, Mannheim.

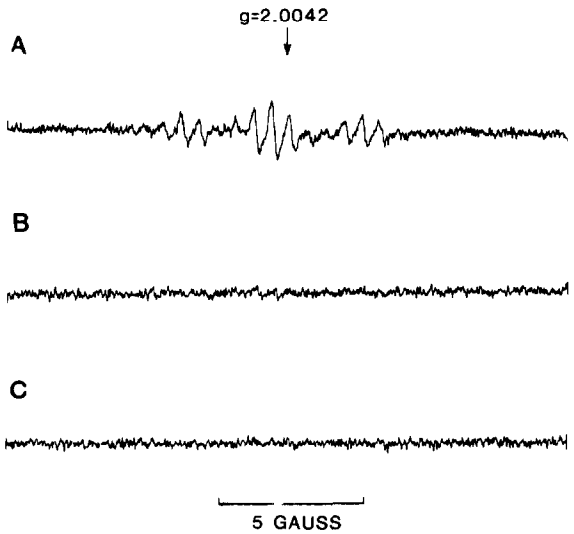

Fig.1. 1,4-Naphthosemiquinone anion radical spectrum generated during the reaction of 1,4-naphthoquinone with GSH. Reactions contained: A, 1,4-NQ (1 mM) and GSH (250 μM); B, 1,4-NQ (1 mM); C, 1,4-NQ (1 mM) and GSSG (125 μM). The results shown are from one experiment typical of three.

All ESR spectra were recorded on a Bruker 200D X-band spectrometer operating at a nominal frequency of 9.76 GHz. The magnetic field was measured accurately with a nuclear magnetic resonance probe and the frequency with an external counter. The sample tube was of 1 mm internal diameter spectrosil.

The quinones, prepared as fresh solutions in DMSO, were mixed with the other reactants in 10 mM Hepes, containing 140 mM KCl and 1 mM DETAPAC, pH 7.5. In initial studies, we observed that BQ (1 mM) and 1,4-NQ (1 mM) reacted with Tris, but not with Hepes buffer, pH 7.5, to generate their respective semiquinone radical anions. Therefore in all subsequent experiments Hepes buffer was used. The final concentration of DMSO was 25% (v/v). The buffer was bubbled with nitrogen before addition of the quinone. The reaction was initiated by addition of the quinone in DMSO. All ESR scans were started 5 min after mixing.

3. RESULTS

3.1. *Reaction of quinones with GSH and GSSG*

The spectrum of the 1,4-naphthosemiquinone anion radical generated from the reaction between 1,4-NQ (1 mM) and GSH (250 μM) is shown in fig.1A, and may be analysed as a triplet (3.19 G) of quintets (0.62 G). These results are similar to those given by Stone and Maki [14] for 1,4-naphthosemiquinone anion radical in aqueous solution, generated by electrochemical reduction. The *g* value of 2.0044 is the same as that reported by Adams et al. [15] for this radical anion. The formation of the radical was dependent on GSH (fig.1B). Under identical conditions, no signal was detected with a similar concentration of GSSG (250 μM GSH equiv.) and 1,4-naphthoquinone (fig.1C).

To determine whether a similar reaction occurred with other quinones, we studied the interaction of GSH with BQ and 5-OH-1,4-NQ. Both these quinones showed formation of their respective semiquinones when reacted with GSH (250 μM) (fig.2C and A). 5-OH-1,4-NQ (1 mM) and 1,4-NQ (1 mM) showed no detectable radical generation with GSSG (125 μM) (fig.2B) whereas BQ (1 mM) reacted with GSSG to form a benzosemiquinone radical (fig.2D) although much

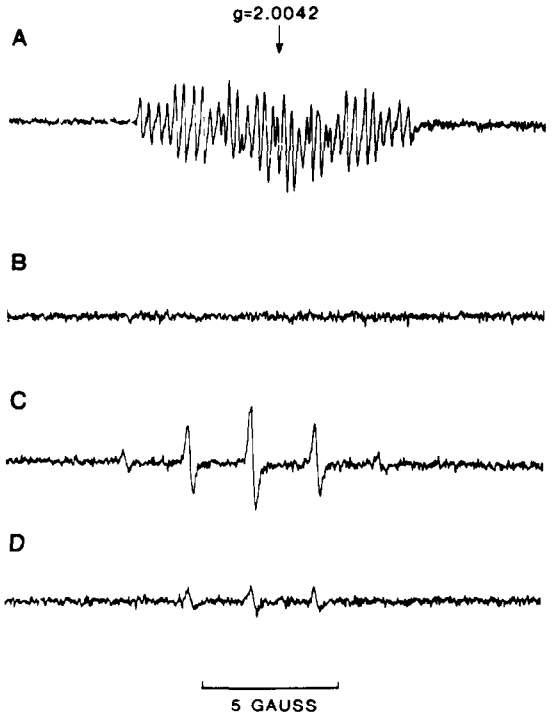

Fig.2. ESR spectra of the semiquinone anion radicals generated by reaction of benzoquinone or 5-OH-1,4-naphthoquinone with GSH or GSSG. Reactions contained: A, 5-OH-1,4-NQ (1 mM) and GSH (250 μM); B, 5-OH-1,4-NQ (1 mM) and GSSG (125 μM); C, BQ (1 mM) and GSH (250 μM); D, BQ (1 mM) + GSSG (125 μM). The results shown are from one experiment typical of three.

reduced compared to the reaction with GSH (fig.2C). The splitting constant for the benzosemiquinone anion radical was calculated to be 2.36 G (4H), similar to that derived by Stone and Maki [14]. The g value of 2.0047 is close to that calculated for the radical anion in a different solvent [15]. The radical anion of 5-OH-1,4-NQ displayed splitting constants of two doublets (0.319 and 0.699 G), one triplet (1.319 G) and two doublets whose splitting constants when summed were 6.30 G. Our spectrum appeared similar to that obtained by Dodd and Mukherjee [16] for this radical in aqueous media.

3.2. *Reactions of quinones with amino acids*

In addition to the reaction of the quinones with thiol groups, we extended our studies to determine whether they would also interact with amino acids to generate semiquinone radicals. BQ (fig.3A and B), 1,4-NQ (fig.3C and D) and 5-OH-1,4-NQ (not shown) reacted with glycine to produce their respective semiquinone radicals, but the signals resulting from these reactions were much smaller than those obtained with GSH under identical conditions (cf. figs 1A and 2C). With a higher concentration of glycine (20 mM), the intensity of the signal was increased (figs 3B and D) indicating a greater radical concentration. BQ (1 mM) also reacted with other amino acids, including alanine (20 mM), arginine (20 mM) and cysteine (250 μM), to form the benzosemiquinone radical (not shown).

To determine whether it was the thiol or the amino group on GSH which was primarily responsible for the reaction with the quinones, the reaction of 1,4-NQ (1 mM) with *N*-acetylcysteine (250 μM) was studied. The 1,4-naphthosemiqui-

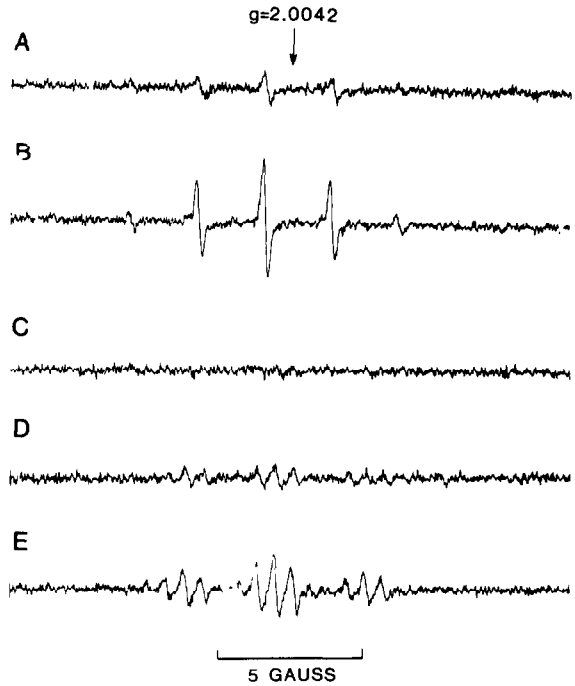

Fig.3. ESR spectra of the semiquinone anion radicals generated by reaction of quinones with glycine or *N*-acetylcysteine. Reactions contained: A, BQ (1 mM) and glycine (250 μM); B, BQ (1 mM) and glycine (20 mM); C, 1,4-NQ (1 mM) and glycine (250 μM); D, 1,4-NQ (1 mM) and glycine (20 mM); E, 1,4-NQ (1 mM) and *N*-acetylcysteine (250 μM). The results shown are from one experiment typical of three.

none, anion radical spectrum obtained (fig.3E) suggested that it was the thiol group of GSH that was mainly undergoing the reaction with the quinone to generate the corresponding semiquinone radical.

4. DISCUSSION

These results indicate that a number of structurally simple quinones, i.e. BQ, 1,4-NQ and 5-OH-1,4-NQ, react with thiol nucleophiles to generate their respective semiquinone radicals (figs 1 and 2). In addition, similar free radicals were observed following the reaction of BQ and 1,4-NQ with the amino group of glycine (fig.3) and other amino acids. This latter reaction occurred much less readily and a greater concentration of glycine (20 mM) compared with GSH (250 μM) was required to detect the radical with 1,4-NQ (cf. fig.1A with fig.3C and D). Although the mechanism of the semiquinone radical production is unclear, and is currently under investigation, one possibility is that the radicals arise following disproportionation of the parent quinone and hydroquinone [17] (eqn 3), the latter having arisen from reactions (1) and (2).

$$Q + HQ \rightleftharpoons 2SQ^{\cdot} \quad (3)$$

Thus the greater production of radicals following reaction of 1,4-NQ with GSH (fig.1A) or *N*-acetylcysteine (fig.3E), compared to glycine (fig.3C and D) can be explained in terms of the chemical hardness and softness of these nucleophiles [18]. The addition reaction (eqn 1) takes place across the double bond between carbons 2 and 3, which can be regarded as a very soft electrophilic site. As soft electrophiles react more readily with soft nucleophiles [18] and the thiol group is much softer than the amino group [19], then it would be expected that quinones would react more readily with the thiol than the amino group.

To determine whether it was the thiol or amino group on the GSH that was primarily responsible for the radical generation with the quinones, 1,4-NQ was reacted with *N*-acetylcysteine (250 μM) (fig.3E). The intensity of the signal obtained was much greater than that achieved with glycine (250 μM) (fig.3C) but the same as that obtained with GSH (250 μM) (fig.1A). This indicates that it is the thiol group in GSH which is primarily responsible for the reaction with the quinones. This is supported by the data obtained with GSSG where no radical generation was seen with 1,4-NQ (fig.1C) and 5-OH-1,4-NQ (fig.2B) whereas with BQ only a small signal was observed (fig.2D) of intensity similar to that obtained with glycine (250 μM) (fig.3A). Glycine (250 μM) has the same concentration of amino groups as GSSG (125 μM).

Semiquinone free radicals have generally been proposed to arise in biological systems by one electron reduction of quinones by cellular reductases such as NADPH-cytochrome P-450 reductase, NADH-cytochrome b_5 reductase or NADH-ubiquinone oxidoreductase [20,21]. The semiquinone radicals are then thought to exert their toxicity either directly or by the generation of active oxygen species and redox cycling [22–25]. In this study, we have shown that following interaction with cellular thiols or amino groups, several simple quinones may give rise to their semiquinone radicals. The biological importance of such reactions remains to be assessed and will depend on several factors including the intracellular concentrations of quinone, availability of thiol and other nucleophilic groups, and the one and two electron reductive metabolism of the quinone. Thus the bone marrow toxicity of benzene and phenol, which is believed to be mediated by a metabolite of phenol such as BQ [26], may in part be due to reactions similar to those described in the current study.

In summary, we have shown that a number of quinones may react with cellular nucleophiles, such as GSH or amino acids, to generate reactive semiquinone radicals. The biological significance of these reactions remains to be assessed.

REFERENCES

[1] Thompson, R.H. (1971) Naturally Occurring Quinones, 2nd edn, Academic Press, London.
[2] Blum, R.H. and Carter, S.K. (1974) Ann. Int. Med. 80, 249–259.
[3] Nickerson, W.J., Falcone, G. and Strauss, G. (1963) Biochemistry 2, 537–543.
[4] Snell, J.M. and Weissberger, A. (1939) J. Am. Chem. Soc. 61, 450–453.
[5] Fieser, L.F. and Turner, R.B. (1947) J. Am. Chem. Soc. 69, 2335–2341.

[6] Blackhall, A. and Thomson, R.H. (1953) J. Chem. Soc. 1138–1143.
[7] Morrison, M., Steele, W. and Danner, D.J. (1969) Arch. Biochem. Biophys. 134, 515–523.
[8] Rothmann, F.G. (1958) J. Org. Chem. 23, 1049–1051.
[9] Nakai, N. and Hase, J. (1968) Chem. Pharm. Bull. 16, 2334–2338.
[10] Schubert, M. (1946) J. Am. Chem. Soc. 69, 712–713.
[11] Finley, K.T. (1974) The Addition and Substitution Chemistry of Quinones, in: The Chemistry of the Quinoid Compounds (Patai, S. ed.) part II, p.878, John Wiley, London.
[12] Meister, A. and Anderson, M.E. (1983) Annu. Rev. Biochem. 52, 711–760.
[13] Ross, D., Thor, M., Orrenius, S. and Moldeus, P. (1985) Chem. Biol. Int. 55, 117–184.
[14] Stone, E.W. and Maki, A.H. (1962) J. Chem. Phys. 36, 1944–1945.
[15] Adams, M., Blois, M.S. and Sands, R.H. (1958) J. Chem. Phys. 28, 774–776.
[16] Dodd, N.J.F. and Mukherjee, T. (1984) Biochem. Pharmacol. 33, 379–385.
[17] Bothner-By, A.A. (1953) J. Am. Chem. Soc. 75, 728–730.
[18] Coles, B. (1984–85) Drug Met. Rev. 15, 1307–1334.
[19] Pearson, R.J. and Songstad, J. (1967) J. Am. Chem. Soc. 89, 1827–1836.
[20] Iyanagi, T. and Yamazaki, I. (1970) Biochim. Biophys. Acta 216, 282–294.
[21] Powis, G. and Appel, P.L. (1980) Biochem. Pharmacol. 29, 2567–2572.
[22] Kappus, H. and Sies, H. (1981) Experientia 37, 1233–1258.
[23] Bachur, N., Gordon, S.L. and Gee, M.W. (1978) Cancer Res. 38, 1745–1750.
[24] Kappus, H. (1986) Biochem. Pharmacol. 35, 1–6.
[25] Thor, H., Smith, M.T., Hartzell, P., Bellomo, G., Jewell, S.A. and Orrenius, S. (1982) J. Biol. Chem. 257, 12419–12425.
[26] Greenlee, W.F., Sun, J.D. and Bus, J.S. (1981) Toxicol. Appl. Pharmacol. 59, 187–195.