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Antioxidant and pro-oxidant properties of pyrroloquinoline quinone (PQQ): implications for its function in biological systems

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Abstract

Pyrroloquinoline quinone (PQQ) is a novel redox cofactor recently found in human milk. It has been reported to function as an essential nutrient, antioxidant and redox modulator in cell culture experiments and in animal models of human diseases. As mitochondria are particularly susceptible to oxidative damage we studied the antioxidant properties of PQQ in isolated rat liver mitochondria. PQQ was an effective antioxidant protecting mitochondria against oxidative stress-induced lipid peroxidation, protein carbonyl formation and inactivation of the mitochondrial respiratory chain. In contrast, PQQ caused extensive cell death to cells in culture. This surprising effect was inhibited by catalase, and was shown to be due to the generation of hydrogen peroxide during the autoxidation of PQQ in culture medium. We conclude that the reactivities of PQQ are dependent on its environment and that it can act as an antioxidant or a pro-oxidant in different biological systems.

Keywords: Pyrroloquinoline quinone (PQQ); Antioxidant; Pro-oxidant; Mitochondria; Redox cycling; Cell death

1. Introduction

Pyrroloquinoline quinone (PQQ) is a non-covalently bound prosthetic group in some bacterial dehydrogenases where it helps catalyze the oxidation of sugars and alcohols [1]. This bacterially-synthesised quinone is a water-soluble and heat-stable molecule that reacts readily with nucleophiles such as amino acids, thiols and ammonia [1]. Although PQQ is not synthesised in mammals it can be rapidly absorbed by the lower intestine after oral administration to young mice, and is subsequently excreted within 24 hr by the kidneys [2]. Trace levels of PQQ have been found in human and rat tissues [3–6], and recently PQQ and its derivative imidazolopyrroloquinoline (IPQ) were found in human milk at concentrations between 140 and 180 ng/mL [7]. This level of PQQ suggests that it may be a micronutrient with biological activity in mammals, although no mammalian PQQ-requiring enzymes have yet been discovered. Supporting a role for PQQ as a micronutrient, PQQ-deficient diets cause impaired growth, immunological defects and decreased fertility in mice [8–10]. While the potential physiological role of PQQ in animals is unclear, it may be acting as an antioxidant: PQQ was shown to scavenge $O_2^{\bullet -}$ and $HO^{\bullet}$ efficiently [11]. A variety of other *in vitro* effects of PQQ have been observed, such as inhibiting lipid peroxidation [12], protecting the isolated heart from reoxygenation injury [13], oxidizing the NMDA receptor redox site [14,15], increasing thymidine incorporation into fibroblasts [16], inhibiting melanogenesis in a murine B16-F10 melanoma [17] and increasing the production of nerve growth factor in cell lines [18,19]. In animal models, high doses of PQQ

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Abbreviations: Ac-DEVD-AMC, *N*-acetyl-Asp-Glu-Val-Asp-aminomethylcoumarin; BHT, butylated hydroxytoluene; CHAPS, 3-cholamidopropyl-dimethylammonio-propanesulfonate; Complex I, NADH-ubiquinone oxidoreductase; DNPH, 2,4-dinitrophenylhydrazine; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; HEPES, *N*-(2-hydroxyethyl)-piperazine-*N*′-(2-ethanesulfonic acid); LDH, lactate dehydrogenase; MOPS, 4-morpholinopropane sulfonic acid; NADH, nicotinamide adenine dinucleotide, reduced form; PQQ, pyrroloquinoline quinone; ROS, reactive oxygen species; TBARS, thiobarbituric acid-reactive substances; Tris, tris[hydroxymethyl]aminomethane.

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protected tissues against hypoxic/ischaemic injury [20], chemical agent-induced seizure [21], carrageenan-induced inflammation [12], liver damage caused by ethanol [22] or carbon tetrachloride [11,23], formation of cataracts and depletion of glutathione in the chicken embryo administrated with glucocorticoids [24,25] and the mortality induced by endotoxin [11]. These protective effects of PQQ seem to be generally attributed to its antioxidant properties [26], but their precise mechanisms are unclear. In addition, PQQ may alter mitochondrial function by lowering respiration rate in mice [27]. Mitochondria are a major source of reactive oxygen species (ROS) within cells, and oxidative damage to mitochondria contributes to a number of degenerative diseases by increasing cell death. Therefore, PQQ may protect mitochondria from oxidative damage and thereby decrease cell death. In this study, we determined whether PQQ affects normal mitochondrial function and tested its antioxidant properties. We found that PQQ was an effective antioxidant against mitochondrial oxidative damage. However, when we extended this work to determine whether PQQ could prevent cell death in cultured cells, we found that PQQ could also act as a pro-oxidant thereby increasing cell death.

2. Materials and methods

2.1. Materials

RPMI 1640 tissue culture medium supplemented with L-glutamine, and heat-inactivated fetal calf serum were from GIBCO-BRL (Life Technologies). Biotinylated *N*-acetyl-Asp-Glu-Val-Asp-aminomethylcoumarin (Ac-DEVD-AMC) was from Calbiochem. PQQ was from Mitsubishi Gas Chemical Co. and stock solutions were prepared in water. All other chemicals used were of analytical reagent grade quality or better and were obtained from the usual commercial sources.

2.2. Mitochondrial incubations

Liver mitochondria from overnight fasted Wistar female rats (150–250 g) were isolated by homogenisation and subsequent differential centrifugation [28] and gently resuspended in the homogenisation medium (STE) consisting of 250 mM sucrose, 10 mM Tris–HCl and 0.1 mM EGTA, (pH 7.4) [29]. For samples subjected to oxidative stress, the final washing and resuspension of mitochondria was in the homogenisation medium without EGTA. Protein concentration was measured by the biuret protein assay using bovine serum albumin as a standard [30].

To induce oxidative stress, mitochondria (4 mg protein) were resuspended in 2 mL KCl–Tris medium (120 mM KCl and 50 mM Tris–HCl, pH 7.4) at 37° or room temperature with gentle shaking [31] and $FeSO_4$ (10 μM) and ascorbate (300 μM) were added. Five microliters 10% BHT in ethanol (0.01% final concentration) was added at the end of the incubation to inhibit further oxidation. Aliquots of the mitochondrial suspension (200 μL) were then removed and lipid peroxidation was measured by the TBARS assay. For protein carbonyls, a 1 mL mitochondrial sample was stored at −80°. To measure mitochondrial respiration rate, the mitochondrial suspension was incubated with $FeSO_4$ and ascorbate as above for 7 min, then pelleted by centrifugation (10,000 *g* for 2 min) and the mitochondrial pellet was gently resuspended in 1 mL KCl–Tris buffer and the respiration rate was measured.

Oxygen consumption rate was measured using a Clark-type oxygen electrode (Rank Brothers, Bottisham, Cambridge, UK) in a magnetically stirred thermostatted chamber in a buffer containing 100 mM KCl, 75 mM mannitol, 25 mM sucrose, 5 mM Tris phosphate (pH 7.4), 10 mM Tris–HCl (pH 7.4), and 0.05 mM EDTA [32]. Mitochondria (2 mg protein) were added to 2 mL of respiration buffer ± PQQ. For the study of mitochondria subjected to oxidative damage, 1 mL mitochondrial suspension (1 mg protein mL^{-1}) was transferred to the chamber along with 1 mL KCl–Tris buffer. The respiratory substrates and inhibitors used were: glutamate and malate (10 mM each) or succinate (10 mM) in the presence of rotenone (10 μM). Mitochondrial respiration was initiated by the addition of substrate, state 3 respiration was induced by the addition 300 μM ADP, which was followed by the state 4 respiration upon depletion of ADP. For uncoupled respiration, 1 μM FCCP was added after state 4 respiration had been attained. The output from the electrode was recorded automatically by a MacLab™ data acquisition system and the rate of oxygen consumption was determined. The respiratory control ratio (RCR) was calculated as the ratio of the state 3 to state 4 respiration [33].

2.3. Cell incubations

Jurkat cells were grown at 37° in a humidified atmosphere of 95% air and 5% CO_2 in RPMI medium 1640 (pH 7.4) supplemented with 100 mg streptomycin, 60 mg penicillin G and 10% fetal calf serum. For experiments, the cells were harvested by centrifugation (800 *g* for 5 min), suspended in fresh medium and adjusted to 10^6 cells mL^{-1}.

Cell viability was estimated from the amount of lactate dehydrogenase (LDH) retained in the cells. LDH (EC 1.1.1.28) activity was measured as the rate of NADH oxidation at 340 nm ($\epsilon_{340} = 6.22 \times 10^3\ M^{-1}\ cm^{-1}$) [34]. To evaluate the toxicity of PQQ, Jurkat cells (1 mL, 1×10^6 cells) were treated with PQQ ± catalase at 37° for 24 hr. At the end of this incubation, cells were pelleted by centrifugation (10,000 *g* for 2 min), resuspended in 100 μL PBS buffer and stored at −20°. After freeze-thawing (×3) and centrifugation (10,000 *g* for 2 min), the supernatants were used to measure LDH activity. To do this, 30 μL supernatant was added to 1 mL KH_2PO_4 buffer (50 mM at pH 7.5) containing 0.63 mM potassium

pyruvate and 0.25 mM NADH at 30°. The oxidation of NADH was monitored using a spectrophotometer. PQQ-induced cytotoxicity is expressed as percentage LDH activity in treated cells compared to vehicle-treated cells.

Apoptosis of Jurkat cells was monitored by measuring caspase-3 activity [35]. Jurkat cells (5 mL, 5×10^6 cells) were incubated with various concentrations of PQQ ± catalase at 37° with gentle shaking. Cells were then pelleted by centrifugation (1000 *g* for 5 min) and either analysed immediately, or snap frozen and stored at −80° for later analysis. Cells were lysed in 100 μL lysis buffer [100 mM HEPES, 10% sucrose, 5 mM dithiothreitol and 0.1% CHAPS at pH 7.25] containing 50 μM Ac-DEVD-AMC and placed in a 96-well microtiter plate. Substrate cleavage to produce fluorescent AMC was monitored continuously at room temperature using a Cary Eclipse fluorescence spectrophotometer (Varian Inc., Mulgrave, Victoria, Australian) with $\lambda_{excitation} = 355$ nm and $\lambda_{emission} = 460$ nm. The caspase-3 activity was calculated from the initial rate of increase in fluorescence intensity using the Cary Eclipse software and data are presented in arbitrary fluorescence units per 10^6 cells min^{-1}.

2.4. Complex I activity

Isolated mitochondria were pelleted by centrifugation and resuspended in ice-cold KCl medium (150 mM KCl, 25 mM $NaHCO_3$, 1 mM $MgCl_2$, 3 mM KH_2PO_4, 20 mM HEPES, pH 7.4) [36] at 10 mg protein mL^{-1}. Then 100 μL mitochondrial suspension was added to 300 μL KCl buffer containing 10 mM each of glutamate and malate (±PQQ) and incubated at room temperature for 1 hr. Since PQQ directly oxidises NADH, interfering with the Complex I assay, the mitochondria were then washed twice with 500 μL KCl buffer, adjusted to 3 mg protein mL^{-1} and aliquots were stored at −80°. Mitochondrial samples were freeze-thawed (×3) and Complex I activity was measured as the rotenone-sensitive rate of NADH oxidation [37,38]. The assay was performed at 30° in 1 mL KCl buffer (50 mM KCl, 10 mM Tris and 1 mM EDTA at pH 7.4) containing 2 mM KCN, 300 nM antimycin, 0.125 mM NADH, and 50 μM ubiquinone-1. The reaction was initiated by the addition of mitochondrial protein (25–50 μg) and the linear decrease in absorbance of NADH was monitored at 340 nm for 2 min. Rotenone (5 μg) was then added and the rotenone-insensitive activity was measured and expressed as nmol NADH min^{-1} mg mitochondrial $protein^{-1}$.

2.5. Oxidative stress assays

Lipid peroxidation in isolated mitochondria was measured from the formation of thiobarbituric acid-reactive substances (TBARS) [39]. The mitochondrial suspension (200 μL) was mixed with 200 μL 1% TBA (dissolved in 50 mM NaOH), 100 μL 20% H_3PO_4, and 20 μL 10 M NaOH. The mixture was heated at 90° for 15 min, rapidly cooled on ice and butanol (750 μL) was added. The mixture was shaken and then centrifuged (2750 *g* for 5 min). TBARS fluorescence of 500 μL of the butanol layer was determined using a fluorescence spectrophotometer ($\lambda_{excitation} = 515$ nm and $\lambda_{emission} = 553$ nm) and the TBARS concentration was expressed as arbitrary fluorescence units per milligram protein.

Protein carbonyls were measured by a spectrophotometric method [40,41]. Two 150 μL samples containing 0.3 mg Fe^{2+}-oxidised mitochondrial protein were analysed in parallel. One was incubated with 600 μL 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2.5 M HCl, the other was incubated with 2.5 M HCl without 2,4-dinitrophenylhydrazine to estimate protein loss during analysis by measuring the protein concentration in the pellets by reading the absorption at 278 nm. Following 1 hr incubation at room temperature with vortexing every 15 min, 750 μL 20% (w/v) trichloroacetic acid (TCA) was added, the samples were placed on ice for 10 min and then precipitated protein was pelleted by centrifugation (11,000 *g* for 5 min). The protein pellet was washed (×4) with 1 mL ethanol:ethyl acetate (1:1, v/v) for 10 min at room temperature, followed by centrifugation as above. The washed protein pellet was then dried at room temperature and dissolved in 1 mL 6 M guanidinium hydrochloride, 20 mM potassium phosphate (pH 2.3) at 37° for 15 min. DNPH derivatives of protein carbonyls were quantified at 375 nm ($\epsilon = 22{,}000\ M^{-1}\ cm^{-1}$) [40].

The production of H_2O_2 in cell-free culture medium (RPMI 1640) was measured using an oxygen electrode [42]. To do this, the oxygen electrode response was stabilised for 30 min with 2 mL air-saturated PBS (pH 7.4) while RPMI 1640 medium was stirred at room temperature for 30 min to saturate with air. The response of the oxygen electrode to oxygen saturated medium was adjusted to 50% full-scale deflection. To create a H_2O_2 calibration curve, 2 mL RPMI 1640, pre-warmed at 37°, was loaded into the oxygen electrode chamber and freshly prepared H_2O_2 was added 30 s later, then catalase (2000 units in 20 μL) was injected to give a spike of O_2 evolution. To measure H_2O_2 production by PQQ, 2 mL RPMI 1640 medium containing 250 μM PQQ was incubated at 37° for designated time periods and then transferred to the oxygen electrode chamber. The concentrations of H_2O_2 in PQQ-containing culture media were measured by the addition of catalase as above and calculated from the H_2O_2 standard curve.

2.6. Statistical analysis

All statistical calculations were carried out using the SPSS software program (version 9.0 for Windows). The data were expressed as means ± standard error of the means (SEM) from at least three independent experiments with different sample preparations. Statistical comparison on the experimental data was analysed with either Student's *t*-test (two-tailed unpaired group) or a one-way ANOVA

test followed by the Bonferroni test, as appropriate. Differences between means were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Effect of PQQ on mitochondrial function

Before analysing any antioxidant effects of PQQ it was first important to determine whether it disrupted mitochondrial function. The enzyme activity of Complex I in PQQ-treated isolated rat liver mitochondria was measured because this enzyme is sensitive to oxidative damage. In an initial experiment, PQQ was found to interfere with the Complex I assay (data not shown) due to its direct oxidation of NADH [43]. Therefore, to avoid artifactual oxidation of NADH, mitochondria were washed after treatment to remove PQQ and then the rotenone-sensitive activity of Complex I was determined. The Complex I activity was not affected by 500 μM PQQ treatment (36.3 ± 2.0 nmol NADH min^{-1} mg $protein^{-1}$) when compared with controls (33.7 ± 7.0 nmol NADH min^{-1} mg $protein^{-1}$). To investigate any direct interference of PQQ with the mitochondrial respiratory chain, the uncoupled respiration rate of isolated mitochondria was measured in the presence of 500 μM PQQ (Fig. 1A). PQQ did not change uncoupled respiration on different substrates. The effects of 500 μM PQQ on coupled mitochondrial respiration and on the respiratory control ratio (RCR) were measured to indicate effects on mitochondrial membrane integrity and ATP synthesis. PQQ did not affect the RCR in mitochondria energised with either glutamate and malate (Fig. 1B), or succinate (Fig. 1C). These experiments indicate that PQQ concentrations up to at least 500 μM are not damaging to mitochondria and can therefore be used to investigate the potential protective properties of PQQ against oxidative damage to mitochondria.

3.2. Antioxidant effects of PQQ on isolated mitochondria

To study the antioxidant properties of PQQ, mitochondria were exposed to oxidative stress by the addition of ferrous iron/ascorbate [44,45]. The level of TBARS was measured as an indicator of lipid peroxidation and this increased significantly on incubation of mitochondria with ferrous iron/ascorbate (Fig. 2A). When mitochondria were incubated with PQQ (2.5–50 μM) and then subjected to this oxidative stress, PQQ significantly prevented lipid peroxidation in a dose-dependent manner (Fig. 2A), with 50 μM PQQ completely inhibiting lipid peroxidation. To eliminate the possibility that inhibition by PQQ was due to interference with the TBARS assay, control experiments were carried out in which PQQ (50 μM) was added to the mitochondria at the end of the incubation which was then

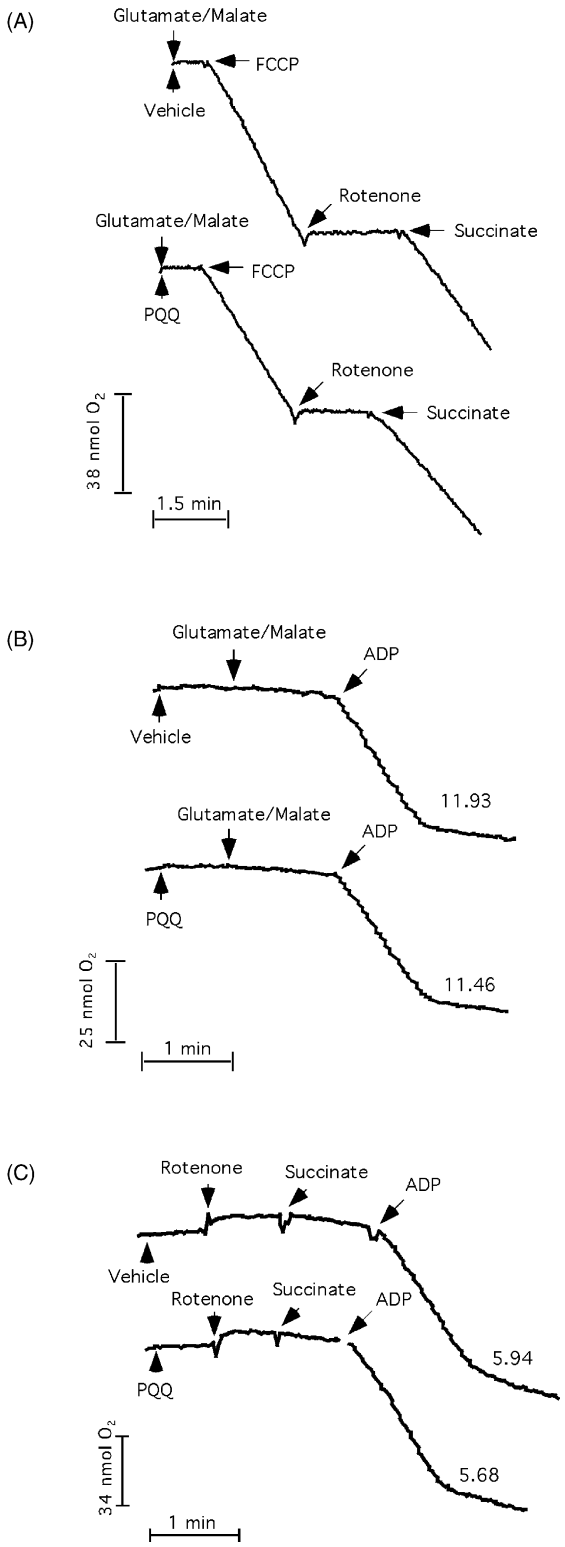

Fig. 1. Mitochondrial respiration in the presence of PQQ. Panel A: mitochondria (2 mg protein) were added into the respiration buffer (2 mL). Where indicated (arrows), 500 μM PQQ (or vehicle control), 10 mM glutamate and 10 mM malate, 1 μM FCCP, 2 μM rotenone and 10 mM succinate were added; Panels B and C: experimental conditions were similar to panel A except that FCCP was omitted. The respiratory substrates are glutamate and malate in panel B and succinate in panel C, and state 3 respiration was stimulated by the addition of ADP (300 μM). The data here are a representative experiment and the numbers along the traces are the RCR measured as described in Section 2.

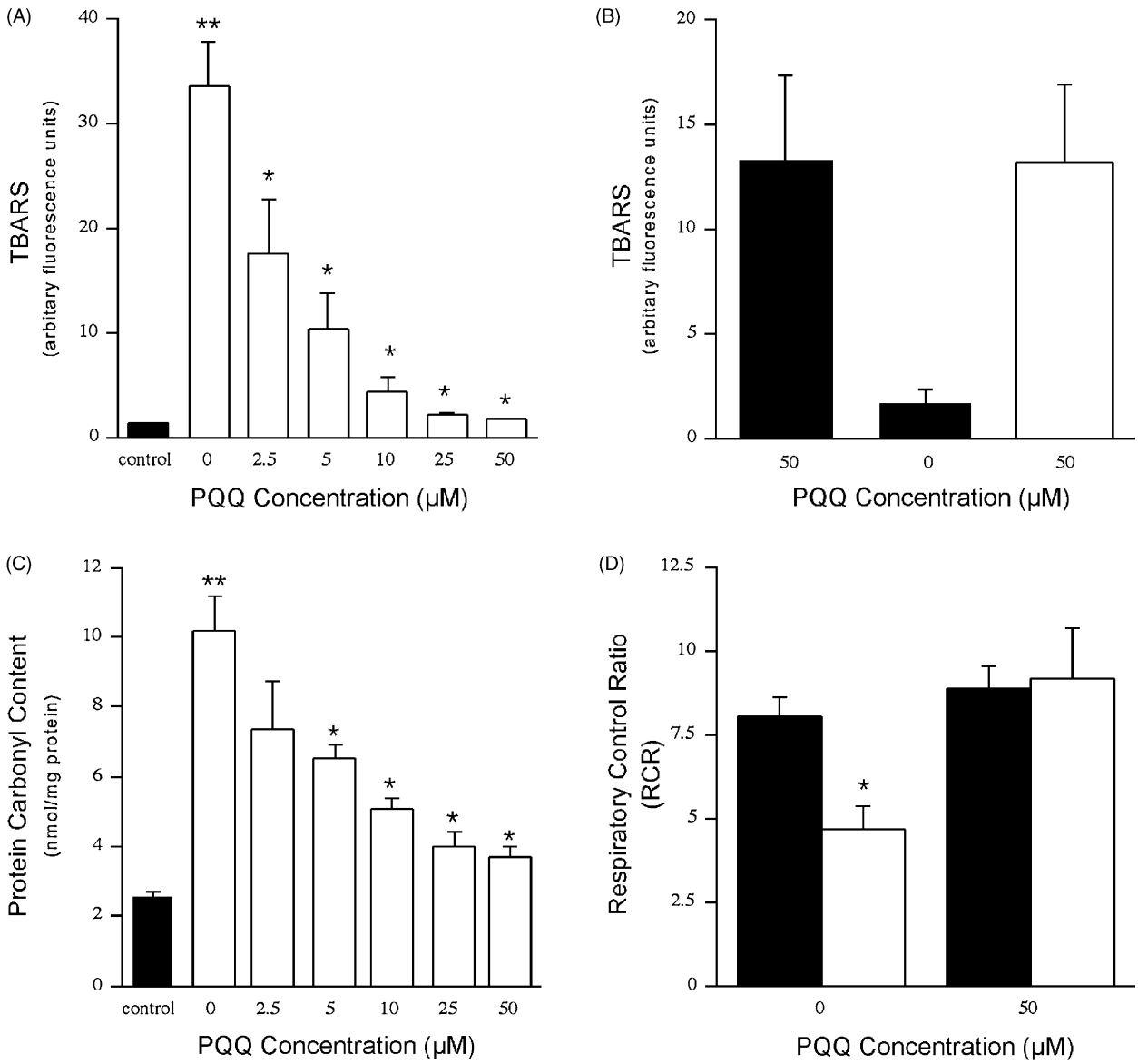

Fig. 2. Antioxidant effect of PQQ in mitochondria exposed to oxidative stress. Panel A shows the effect of PQQ on lipid peroxidation induced by $FeSO_4$ (10 μM) and ascorbic acid (300 μM) at 37° for 30 min. Mitochondria were energized with glutamate and malate (10 mM each) in the presence of PQQ (2.5–50 μM). Control samples (filled bar) were not treated with either Fe^{2+}/ascorbate or with PQQ. $^{**}P < 0.05$, compared with control samples (filled bar) and $^{*}P < 0.05$, compared with oxidatively stressed samples without PQQ treatment; Panel B: the effect of the timing for addition of PQQ on lipid peroxidation. Experimental conditions were the same as in panel A. Open bar indicates that mitochondrial samples were treated with PQQ at the completion of a 30 min incubation with iron, whereas for the other (filled bars) PQQ was added at the beginning of the incubations. The data are from two independent experiments; Panel C: the effect of PQQ on protein carbonyl formation in mitochondria. $^{**}P < 0.05$, compared with untreated controls (filled bar), $^{*}P < 0.05$, compared with oxidatively stressed samples without PQQ treatment; Panel D: PQQ protection against the effect of oxidative stress on mitochondrial coupling. Sample preparation and the measurement of state 3 and state 4 respiratory rates were described in Section 2. Open bars show data from mitochondrial samples treated with $FeSO_4$, whereas non-Fe^{2+} treated samples shown as filled bars. $^{*}P < 0.05$, compared to control samples without $FeSO_4$ treatment (filled bar, 0 μM).

processed as before. This control showed that the level of TBARS was the same as for mitochondria which had not been treated with PQQ (Fig. 2B), indicating that PQQ did not affect the TBARS measurement.

The effect of PQQ on protein carbonyl formation, an indicator of oxidative damage to proteins, was then investigated. Mitochondrial samples treated with ferrous iron significantly increased their protein carbonyl content, and addition of PQQ decreased protein carbonyl formation in a dose-dependent manner, with 50 μM PQQ inhibiting protein carbonyl formation by more than 85% (Fig. 2C). Finally, to determine whether the antioxidant activity of PQQ protected mitochondrial function, the respiratory control ratio (RCR) of mitochondrial samples was measured. As seen in Fig. 2D, incubation with ferrous iron decreased the RCR significantly by ~42% to 4.64 ± 0.68 compared to the control value of 8.02 ± 0.55. In contrast, PQQ treatment completely protected mitochondria from this oxidative damage, with the RCR of PQQ-treated mitochondria (8.85 ± 0.65) being indistinguishable from

controls (8.32 ± 0.43). This protection by PQQ was due to prevention of both the increase in state 4 respiration and the decrease in state 3 respiration on exposure to ferrous iron. Thus, PQQ prevents oxidative damage to mitochondrial lipid and proteins, and also protects the physiological function of the organelle from oxidative damage.

3.3. Pro-oxidant effects of PQQ on cells in culture

PQQ was effective in protecting mitochondria from oxidative damage. As mitochondrial dysfunction contributes to cell death we were interested to see whether PQQ would also prevent oxidant-induced cell death. To do this, we used Jurkat cells, which are a widely used model for oxidant-induced cell death [35,46]. To our surprise, when we treated Jurkat cells with PQQ it was toxic to the cells and after 24 hr incubation there was clear concentration-dependent cytotoxicity (Fig. 3A). Significantly, the concentrations that caused extensive cell death (50–250 μM PQQ) did not damage isolated mitochondria and were in fact protective against oxidative damage. Caspase-3 activation, a hallmark of apoptosis and a more sensitive marker

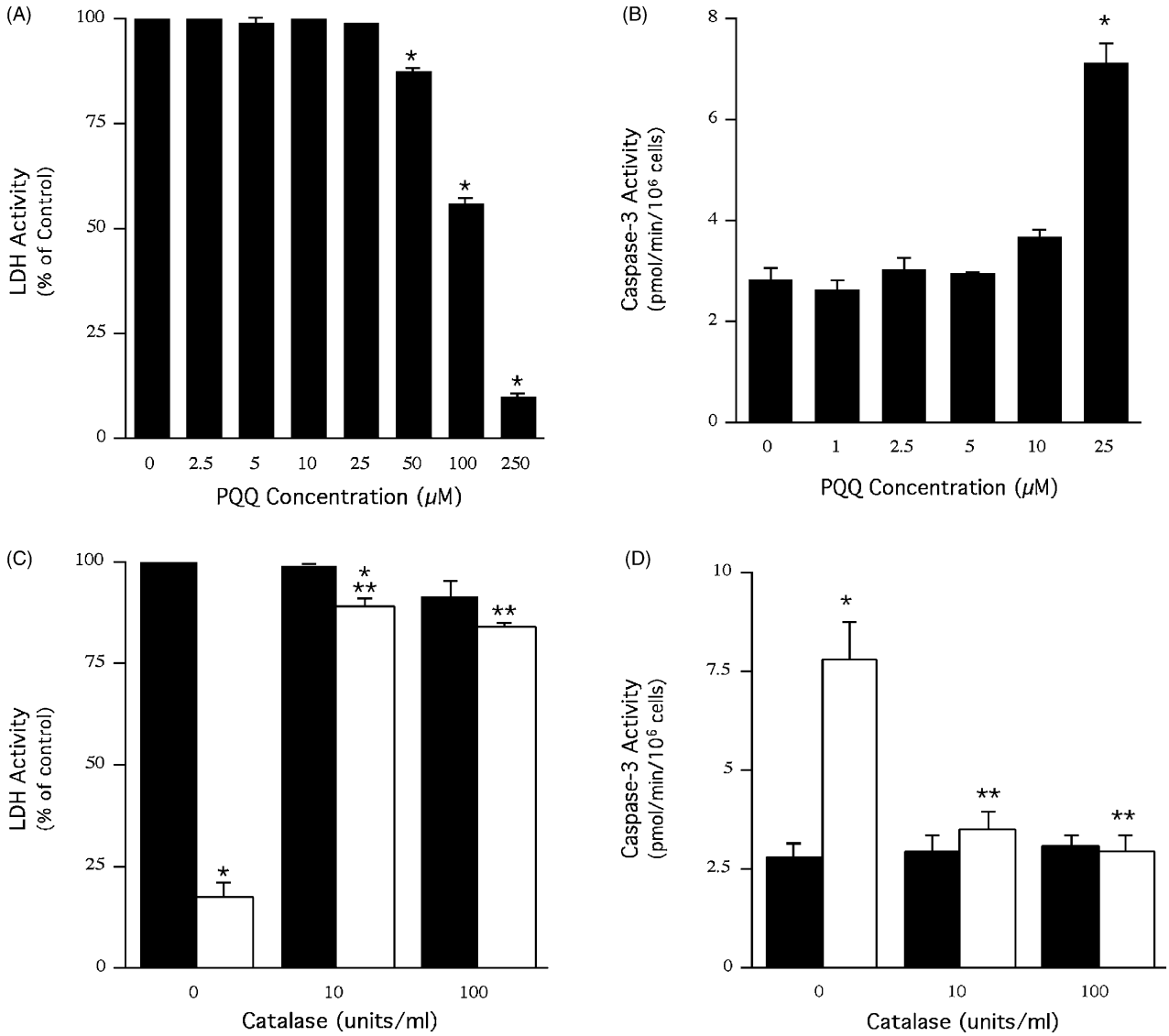

Fig. 3. Hydrogen peroxide is responsible for PQQ-induced Jurkat cell death. Panel A: PQQ caused concentration-dependent cell death after 24 hr incubation by the measurement of the loss of intracellular LDH activity. $^{*}P < 0.05$ is for comparison with untreated control samples; Panel B: PQQ (25 μM) induced apoptotic cell death after incubation at 37° for 5 hr and increased caspase-3 activity was detected as described in Section 2. $^{*}P < 0.05$ is for comparison with untreated control samples; Panel C: Inhibitory effect of catalase on PQQ-induced cytotoxicity in Jurkat cells. Jurkat cells were cultured with PQQ (250 μM) ± catalase for 24 hr and then the intracellular LDH activity was measured. Data are a percentage of the total amount of LDH present in control Jurkat cells (filled bars labelled zero). Open bars represent Jurkat cells treated with PQQ, filled bars are not treated with PQQ. $^{*}P < 0.05$ is for comparison with untreated controls (vehicle, filled bar) and $^{**}P < 0.05$ is for comparison with control samples treated with PQQ only (open bar); Panel D: the protection by catalase on PQQ-induced apoptosis. Jurkat cells were incubated with PQQ (25 μM) ± catalase at 37° for 5 hr and then caspase-3 activity was measured. Open bars represent Jurkat cells treated with PQQ, filled bars are for cells not treated with PQQ. $^{*}P < 0.05$ is for comparison with untreated controls (vehicle, filled bars), whereas $^{**}P < 0.05$ is for comparison with control samples treated with PQQ only (open bars).

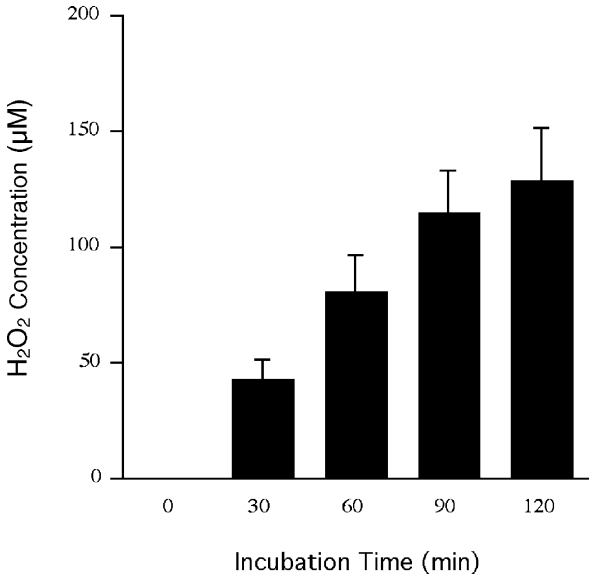

Fig. 4. Time-dependent generation of hydrogen peroxide in PQQ-containing RPMI 1640 medium. PQQ (250 μM) was added to the RPMI 1640 medium and incubated at 37° for various time periods (0–120 min). At the designated time points, the medium was collected and the level of hydrogen peroxide was measured using the assay as described in Section 2. No hydrogen peroxide was detected at the beginning of the PQQ incubation (0 min).

of cell death than LDH release, was also measured after treating Jurkat cells with low concentrations of PQQ (1–25 μM) (Fig. 3B). At 25 μM PQQ, there was 2.7-fold increase in caspase-3 activity compared with controls. This toxicity of PQQ was in contrast to the protective effects of PQQ with isolated mitochondria. We suspected that PQQ might be producing hydrogen peroxide as an end product of PQQ redox cycling. This was confirmed by showing that the hydrogen peroxide scavenger catalase protected cells from PQQ toxicity (Fig. 3C and D). Furthermore, treating Jurkat cells with hydrogen peroxide (25–100 μM) for 5 hr caused similar levels of apoptotic cell death as that induced by PQQ (data not shown). We found that hydrogen peroxide production by PQQ-containing RPMI 1640 medium was time-dependent and that its concentration reached 125 μM after 120 min (Fig. 4). These data indicate that PQQ interacts with the cell culture medium to generate hydrogen peroxide and that the PQQ-induced cell death was due to the extracellular generation of this oxidant. However, other mechanisms could also contribute to cell death, such as the production of superoxide, and these possibilities cannot be excluded. As PQQ can react with thiols [47,48], amines, amino acids [7,49,50] and NAD(P)H [51,52] to produce hydrogen peroxide the cell culture component responsible for this reaction is uncertain.

3.4. Summary

Here we have tested the effects of PQQ on Complex I activity and mitochondrial function and found that these were unaffected up to at least 500 μM PQQ. We then investigated the antioxidant effects of PQQ in isolated mitochondria subjected to oxidative stress and found that lipid peroxidation and protein carbonyl formation were significantly inhibited by PQQ. PQQ also protected mitochondrial respiratory function from oxidative inactivation. However, when PQQ was tested in oxidant-sensitive Jurkat cells these cells underwent cell death due to PQQ reacting with components of the culture medium to generate toxic levels of hydrogen peroxide. Therefore, hydrogen peroxide production may contribute to PQQ toxicity in cell culture experiments, unless scavengers such as catalase are used.

In summary, we have shown that PQQ can be either an antioxidant or a pro-oxidant, dependent on the experimental conditions. PQQ is non-toxic to isolated mitochondria and is an effective antioxidant, consistent with a role for PQQ as an antioxidant *in vivo*.

Acknowledgments

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