Free Radical Biology and Medicine 182 (2022) 119–131

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Coenzyme Q_{10} and related quinones oxidize H_2S to polysulfides and thiosulfate

Kenneth R. Olson [a,b,*], Kasey J. Clear [c], Paul J. Derry [d], Yan Gao [a], Zhilin Ma [a,b], Gang Wu [d], Thomas A. Kent [e,f,g], Karl D. Straub [h,i]

[a] Indiana University School of Medicine - South Bend Center, South Bend, IN, 46617, USA
[b] Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, 46556, USA
[c] Department of Chemistry and Biochemistry, Indiana University South Bend, South Bend, IN, 46615, USA
[d] Department of Internal Medicine, University of Texas - McGovern Medical School at Houston, Houston, TX, 77030, USA
[e] Center for Genomics and Precision Medicine, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX, 77030, USA
[f] Department of Chemistry, Rice University, Houston, TX, 77005, United States
[g] Stanley H. Appel Department of Neurology, Houston Methodist Hospital and Research Institute, 6560 Fannin Street, Houston, TX, 77030, United States
[h] Central Arkansas Veteran's Healthcare System, Little Rock, AR, 72205, USA
[i] Departments of Medicine and Biochemistry, University of Arkansas for Medical Sciences, Little Rock, AR, 72202, USA

ARTICLE INFO

Keywords:
Reactive sulfur species
Reactive oxygen species
Antioxidants
CoQ_{10}
Down syndrome

ABSTRACT

In the canonical pathway for mitochondrial H_2S oxidation electrons are transferred from sulfide:quinone oxidoreductase (SQR) to complex III via ubiquinone (CoQ_{10}). We previously observed that a number of quinones directly oxidize H_2S and we hypothesize that CoQ_{10} may have similar properties. Here we examine H_2S oxidation by CoQ_{10} and more hydrophilic, truncated forms, CoQ_1 and CoQ_0, in buffer using H_2S and polysulfide fluorophores (AzMC and SSP4), silver nanoparticles to measure thiosulfate ($H_2S_2O_3$), mass spectrometry to identify polysulfides and O_2-sensitive optodes to measure O_2 consumption. We show that all three quinones concentration-dependently catalyze the oxidization of H_2S to polysulfides and thiosulfate in buffer with the potency CoQ_0>CoQ_1>CoQ_{10} and that CoQ_0 specifically oxidizes H_2S to per-polysulfides, $H_2S_{2,3,4}$. These reactions consume and require oxygen and are augmented by addition of SOD suggesting that the quinones, not superoxide, oxidize H_2S. Related quinones, MitoQ, menadione and idebenone, oxidize H_2S in similar reactions. Exogenous CoQ_0 decreases cellular H_2S and increases polysulfides and thiosulfate production and this is also O_2-dependent, suggesting that the quinone has similar effects on sulfur metabolism in cells. Collectively, these results suggest an additional endogenous mechanism for H_2S metabolism and a potential therapeutic approach in H_2S-related metabolic disorders.

1. Introduction

The seminal study by Hideo Kimura and his group twenty-five years ago showing that hydrogen sulfide (H_2S) is a physiologically relevant endogenous signaling molecule in the nervous system [1] created, essentially de novo, a new field in biology and medicine to the extent that H_2S and related sulfur compounds are now well recognized for their signaling and cytoprotective functions in most organ systems. Most endogenously generated H_2S is derived from cysteine and methionine by cytoplasmic enzymes cystathionone β-synthase (CBS) and cystathionine γ-lyase (CSE, CGL) and by the tandem enzymes, cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfur transferase (3-MST) in both the cytosol and mitochondrion [2,3].

Much of the catabolism of H_2S is believed to occur in the mitochondrion where H_2S is initially oxidized to sulfane sulfur in a reaction catalyzed by sulfide:quinone oxidoreductase (SQR), a monomeric protein in the inner mitochondrial membrane. In the initial oxidation step, H_2S is bound to SQR and two electrons are transferred from H_2S to ubiquinone (coenzyme Q; CoQ_{10}) via FAD; a process recently clarified by a detailed X-ray crystallographic study of human SQR [4]. CoQ_{10} then feeds the electrons into complex III of the electron transport chain (ETC) where they ultimately reduce oxygen at complex IV and contribute to

* Corresponding author. Indiana University School of Medicine -South Bend Raclin Carmichael Hall, 1234 Notre Dame Avenue. South Bend, IN, 46617, United States.
E-mail address: kolson@nd.edu (K.R. Olson).

https://doi.org/10.1016/j.freeradbiomed.2022.02.018
Received 21 November 2021; Received in revised form 3 February 2022; Accepted 18 February 2022
Available online 22 February 2022

ATP synthesis [5,6]. The sulfane sulfur is transferred from SQR to a mobile carrier such as glutathione or sulfite forming glutathione persulfide (GSH-SS) and thiosulfate ($H_2S_2O_3$), respectively and ultimately oxidized to sulfate; the major excretory metabolite of H_2S [4,7]. H_2S oxidation by SQR is believed to be the initial step that commits H_2S to metabolic degradation.

We have recently shown that polyphenolic nutraceuticals in tea, berries and spices catalyze the oxidation of H_2S to polysulfides and thiosulfate [8,9]. This reaction requires oxygen and appears to be mediated by initial autoxidation of a hydroquinone in the B ring of the polyphenol. We have also shown that a variety of quinones and hydroquinones, after the latter are autoxidized, oxidize H_2S to polysulfides and thiosulfate in buffer and in cells [10]. Although side-chain modifications can augment catalytic efficacy, in general, para-quinones are more efficacious than ortho-quinones in catalyyzing H_2S oxidation, and meta-quinones are completely ineffective [10].

CoQ_{10} is also a para-quinone and we hypothesize that it may directly oxidize H_2S independent of SQR. We also hypothesize that other compounds either related to CoQ_{10} or administered to purportedly achieve similar antioxidant effects also metabolize H_2S. Here we show that this is indeed the case. CoQ_{10}, its more water-soluble derivatives CoQ_1 and CoQ_0, the mitochondrial-targeted MitoQ, as well as menadione (vitamin K_3) and idebenone, catalyze H_2S oxidation to polysulfides and thiosulfate ($H_2S_2O_3$). These reactions require O_2 but are independent of SQR. Furthermore, application of CoQ_0 to either HEK293 or HT29 cells decreases endogenous H_2S and increases both polysulfide and thiosulfate production, suggesting that CoQ_0 catalyzes similar processes in cells. These results provide a novel and alternative pathway for H_2S catabolism that requires O_2 but can function independent of SQR and the ETC and therefore will not produce ATP. The cytoprotective attributes of the polysulfides produced by these reactions also suggest that this is an effective therapeutic pathway for future exploration.

2. Methods

2.1. H_2S and polysulfide measurements in buffer

Reagents were aliquoted into 96-well plates and the plates were covered with tape to reduce H_2S volatilization. H_2S was added as Na_2S. Fluorescence was measured with a SpectraMax M5e plate reader (Molecular Devices, Sunnyvale, CA). Excitation/emission (Ex/Em) wavelengths for 7-azido-4-methylcoumarin (AzMC) and 3′,6′-Di(O-thiosalicyl)fluorescein (SSP4) were 365/450 and 482/515 nm, respectively per manufacturer's recommendations. Previous studies have shown that these fluorophores have sufficient specificity relative to other sulfur compounds and reactive oxygen and nitrogen species (ROS and RNS, respectively) to effectively identify H_2S (AzMC) and per- and polysulfides (H_2S_2 and H_2S_n where n = 3–7 or RS_nH where n>1 or RS_nR = where n>2) [11–14]. It should be noted that both AzMC and SSP4 are irreversible and as such provide a historical record of H_2S and polysulfide production and do not reflect cellular concentrations at any specific time.

2.2. Oxygen dependency of polyphenol reaction with H_2S in buffer

To determine if CoQ_0 or CoQ_1 were autoxidized prior to reacting with H_2S, phosphate buffered saline (PBS) was bubbled with either 21% O_2, balance N_2 or 100% N_2 and placed 200 μL into each well of a 96 well plate. Various concentrations of CoQ_0 or CoQ_1 were then added followed by 300 μM H_2S and SSP4 and fluorescence was monitored for 150–180 min. In paired samples, 0.1 μM of superoxide dismutase was added after the CoQ_0 or CoQ_1 but before H_2S.

To confirm that O_2 was consumed in the reaction, O_2 consumption was measured while adding CoQ_0 to H_2S. Oxygen was monitored in a stirred 1 ml water-jacketed chamber at room temperature with a FireStingO$_2$ oxygen sensing system (Pyroscience Sensor Technology, Aachen, Germany) using a non-oxygen consuming 3 mm diameter OXROB10 fiberoptic probe. The probe was calibrated with room air (21% O_2) and 100% nitrogen (0% O_2). H_2S and CoQ_0 were added at timed intervals and percent O_2 (100% equals room air) was measured every 3 s for at least 60 min. Oxygen tension appeared to decrease exponentially after an initial rapid, and linear decrease in O_2. To determine if CoQ_0 was consumed in the reaction and, therefore, the limiting factor in another set of experiments we added a second bolus of 300 μM H_2S and measured O_2 consumption for an additional hour.

2.3. Oxygen-dependency of CoQ_0-catalyzed H_2S consumption

H_2S can be measured in real time in buffer with amperometric sensors. To show that CoQ_0 catalytically removed H_2S and that this was O_2-dependent H_2S was measured amperometrically in PBS in 21% O_2, with or without CoQ_0, and with CoQ0 in 0% O_2. The same experimental setup described above in section 2.2 was employed using an amperometric H_2S sensor constructed in our laboratory. In a typical experiment at 21% O_2, four aliquots of H_2S were added at 10 min intervals to room air-equilibrated PBS or PBS containing 10 μM CoQ_0, and H_2S was continuously measured. For the 0% O_2 experiments PBS containing 10 μM CoQ_0 was exposed to 100% N_2 gas in the chamber until O_2 (monitored with the Firesting optode) approached 0%, at which time the H_2S was added.

2.4. Thiosulfate production

Thiosulfate was measured using silver nanoparticles (AgNP) as described previously [14]. Briefly, AgNPs were prepared by reducing $AgNO_3$ with TA in the presence of $HAuCl_4.4H_2O$. One mL of 20 mM $AgNO_3$ was mixed with 200 μL of 0.5 mM in 98 mL of Milli-Q water at room temperature. One mL of 5.0 mM tannic acid was added and mixture was vigorously stirred, turning yellow within 30 min. The AgNPs were stored at 4 °C until use.

For measurements in PBS, CoQ_0 was incubated with either H_2S (as Na_2S) or mixed polysulfides (as K_2S_n) in 96 well plates and the plates were covered with tape for 60 min to minimize H_2S volatilization during the reaction, then uncovered to allow excess H_2S to dissipate. Thirty μL was then added to 200 μL of the AgNP in 96-well plates and absorbance measured after 60 min at 419 nm. Thiosulfate production by cells was measured at the end of the experiment by removing 30 μL of medium from the well-plates and assaying as above. Absorbance was compared to thiosulfate standards made in either buffer or cell medium and the concentrations plotted against $(A_0\text{-}A)/A_0$ where A_0 and are absorbances of AgNPs without and with thiosulfate, respectively. We note that the standard curve is non-linear but reproducible.

2.5. Absorbance spectra

CoQ_0 absorbance spectra were measured with an Agilent HP 8453 spectrometer (Agilent Technologies, Santa Clara, CA) during reaction with H_2S as NaSH, at several CoQ_0:H_2S concentration ratios and in both aerobic and anaerobic conditions. In initial aerobic experiments 470 μM CoQ_0 was diluted in PBS buffer, pH 7.4 and H_2S (as NaSH) in PBS was added to final concentration of 470 μM. In anaerobic experiments, 470 μM CoQ_0 in PBS buffer, pH 7.4 was placed in a tonometer and the solution was then made anaerobic though 5 cycles of 30 s vacuum followed by 4.5 min of argon gas replacement. NaHS stock solution in PBS buffer was made anaerobic by N_2 gas replacement. The desired volume of H_2S was added to the anaerobic CoQ_0 solution using an air-tight syringe. Spectral changes were followed for 1800 s.

Im other experiments, H_2S (as Na_2S) absorbance spectra were obtained on a Shimadzu UV-2401PC recording spectrophotometer with slit width of 0.5 nm and data points taken at 0.5 nm. Cuvettes were quartz with screw tops with septa and needles were used for inlet and outlet gassing with nitrogen or air. Samples were dissolved in 200 mM PBS, pH

7.39.

2.6. Mass spectrometry

Inorganic polysulfides were identified using liquid chromatography electrospray ionization high resolution mass spectrometry (LC-ESI-HRMS) with several modifications of previously published methods [8]. In a typical experiment, 1 mM H_2S (as Na_2S) was incubated with 10 or 100 μM CoQ0 or menadione in PBS (pH 7.4) and samples removed at approximately 2, 10, 30 and 60 min and derivatized with 10 mM iodoacetamide (IAM). After 1 h, the derivatized samples were detected by LC-ESI-HRMS analysis using a micrOTOF-Q II Mass Spectrometer (Bruker Daltronics) coupled to an UltiMate 3000 (Thermo Fisher) UHPLC system. A Waters Acquity UPLC HSS T3 column (1.8 μm, 150 mm × 2.1 mm inner diameter) was used with mobile phases A (water containing 0.1% formic acid) and B (acetonitrile containing 0.1% formic acid). Samples were diluted 10-fold in water and 20 μL was injected with a linear gradient (0–90% B, 30 min) at a flow rate of 0.4 mL/min. A sample of K_2S_x (mixture of polysulfides) was derivatized with IAM and used to determine chromatographic behavior of the adducts. The mass spectrometer was used in the positive ion mode with the capillary voltage set to 2200 V and drying gas set to 8.0 L/min at 180°C. The IAM polysulfide adducts were detected as the $[M+Na]^+$ ion using their exact masses ±0.002 *m/z*: S_1 (171.0199); S_2 (202.9919); S_3 (234.9640); S_4 (266.9361).

2.7. Cells

Human embryonic kidney (HEK293) or human colon adenocarcinoma (HT29) cells were cultured in T-25 tissue culture flasks and maintained at 37 °C in a 5% CO_2 humidified incubator with 21% O_2 supplemented with DMEM (low glucose) containing 10% FBS and 1% Pen/Strep. They were transferred to 96-well plates and experiments were conducted when cells were 80–95% confluent. Fluorescence was measured on the SpectraMax M5e plate reader as described above. Compounds of interest were typically added after an initial baseline reading.

HEK293 cell experiments were conducted at different O_2 tension to determine if cellular O_2 tension affected sulfur metabolism after addition of CoQ_0 to the cells. Cells were added to 96-well plates with gas-permeable bottoms (Coy Laboratory Products, Inc. Grass Lake, MI) and grown to 80–95% confluency. AzMC or SSP4 and various amounts of CoQ_0 were added to the wells and after an initial baseline reading one plate was returned to the tissue incubator (21% O_2) while the other plate was placed in a model 856-HYPO hypoxia chamber (Plas Labs, Inc. Lansing, MI) and incubated in 5% O_2/5% CO_2 (balance N_2) at 37 °C. The plates were removed at timed intervals, fluorescence measured, and the plates were returned to their respective environments. To minimize excess O_2 exposure, fluorescence from cells in the hypoxia chamber was only measured at 1, 4 and 22 h. A 30 μL sample of the medium was collected after the last fluorescence reading to measure thiosulfate.

2.8. Chemicals

SSP4 was purchased from Dojindo molecular Technologies Inc. (Rockville, MD). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or ThermoFisher Scientific (Grand Island, NY). $>H_2S$ = is used throughout to denote the total sulfide (sum of H_2S + HS^-) derived from Na_2S as S^{2-} most likely does not exist under these conditions [15]. Phosphate buffered saline (PBS; in mM): 137 NaCl, 2.7 KCl, 8 Na_2HPO_4, 2 NaH_2PO_4. Phosphate buffer for absorbance measurements (PB; in mM): 200 Na_2PO_4. pH was adjusted with 10 mM HCl or NaOH to pH 7.4. Stock solutions of CoQ_1 and CoQ_{10} were prepared in ethanol. Chemical structures of CoQ_0, CoQ_1, CoQ_{10}, MitoQ, menadione and idebenone are shown in Fig. 1.

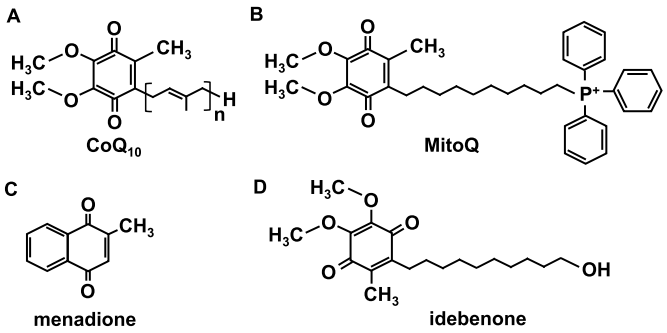

Fig. 1. Chemical structures of CoQ_0, CoQ_1, CoQ_{10}, MitoQ, menadione and idebenone. n indicates the number of isoprenyl units in CoQ.

2.9. Statistical analysis

Data was analyzed and graphed using QuatroPro (Corel Corporation, Ottawa Ont, Canada) and SigmaPlot 13.0 (Systat Software, Inc., San Jose, CA). Statistical significance was determined with Students t-test or one-way ANOVA and the Holm-Sidak test for multiple comparisons as appropriate using SigmaStat (Systat Software, San Jose, CA). Results are given as mean ± SE; significance was assumed when $p < 0.05$.

3. Results

3.1. CoQ_{10}, CoQ_1 and CoQ_0 oxidize H_2S to polysulfides

We have previously shown that benzoquinone and orthoquinone catalytically oxidize H_2S to polysulfides and that the dihydroxyquinones do the same once they have been autoxidized [10]. This suggests that ubiquinone may have similar catalytic properties. To examine this, we incubated CoQ_{10}, CoQ_1 and CoQ_0 with 300 μM H_2S and measured polysulfide production with the polysulfide-specific fluorophore, SSP4. All quinones concentration-dependently increased polysulfide production with an apparent threshold of 1 μM for CoQ_{10} and 0.1 μM for CoQ_1 and CoQ_0 (Fig. 2A–C). The order of potency for polysulfide production was $CoQ_0 > CoQ_1 > CoQ_{10}$. Polysulfide production with 10 and 100 μM CoQ_{10} appeared to lag behind that of 1 mM CoQ_{10} for the initial 75 min then equal it by 90 min, possibility due to the slow dissolution of CoQ_{10}. Fluorescence of SSP4 due to polysulfide production catalyzed by 100 μM CoQ_1 and CoQ_0 plateaued at around 4000 arbitrary units (AUs) within the first 20-30- min and was surpassed by lower quinone concentrations. It is likely that 10 and 100 μM CoQ_{10} would have exceeded the 1 mM response if the CoQ_{10} experiment was extended.

We then examined the catalytic properties of 0, 1 and 10 μM CoQ_1 and CoQ_0 at different H_2S concentrations (Supplemental Fig. S1). H_2S concentration-dependently increased polysulfide production and CoQ_0 was considerably more efficacious at all concentrations of H_2S. The apparent H_2S threshold for either CoQ_1 or CoQ_0 was around 10 μM. More than half of the total SSP4 fluorescence produced by CoQ_0 oxidation of either 300 μM or 1 mM H_2S was achieved by the time the first sample was analyzed (10 min) and these reactions were essentially completed by 30 min.

3.2. CoQ_0 oxidizes H_2S to $H_2S_{(2\text{-}4)}$ polysulfides and thiosulfate

Mass spectrometric analysis of individual polysulfides produced by incubating 1 mM H_2S with either 10 or 100 μM CoQ_0 are shown in Fig. 3A–D. Incubation with 100 μM CoQ_0 produced H_2S_2 H_2S_3 and H_2S_4, whereas only H_2S_2 was present at detectable levels with 10 μM CoQ_0. All polysulfides appeared essentially immediately, H_2S_2 slowly decreased thereafter while H_2S_4 remained relatively constant; H_2S_3 transiently, and inexplicably increased at 30 min with 10 μM CoQ_0, whereas it remained constant with 100 μM CoQ_0. H_2S alone also decreased in the

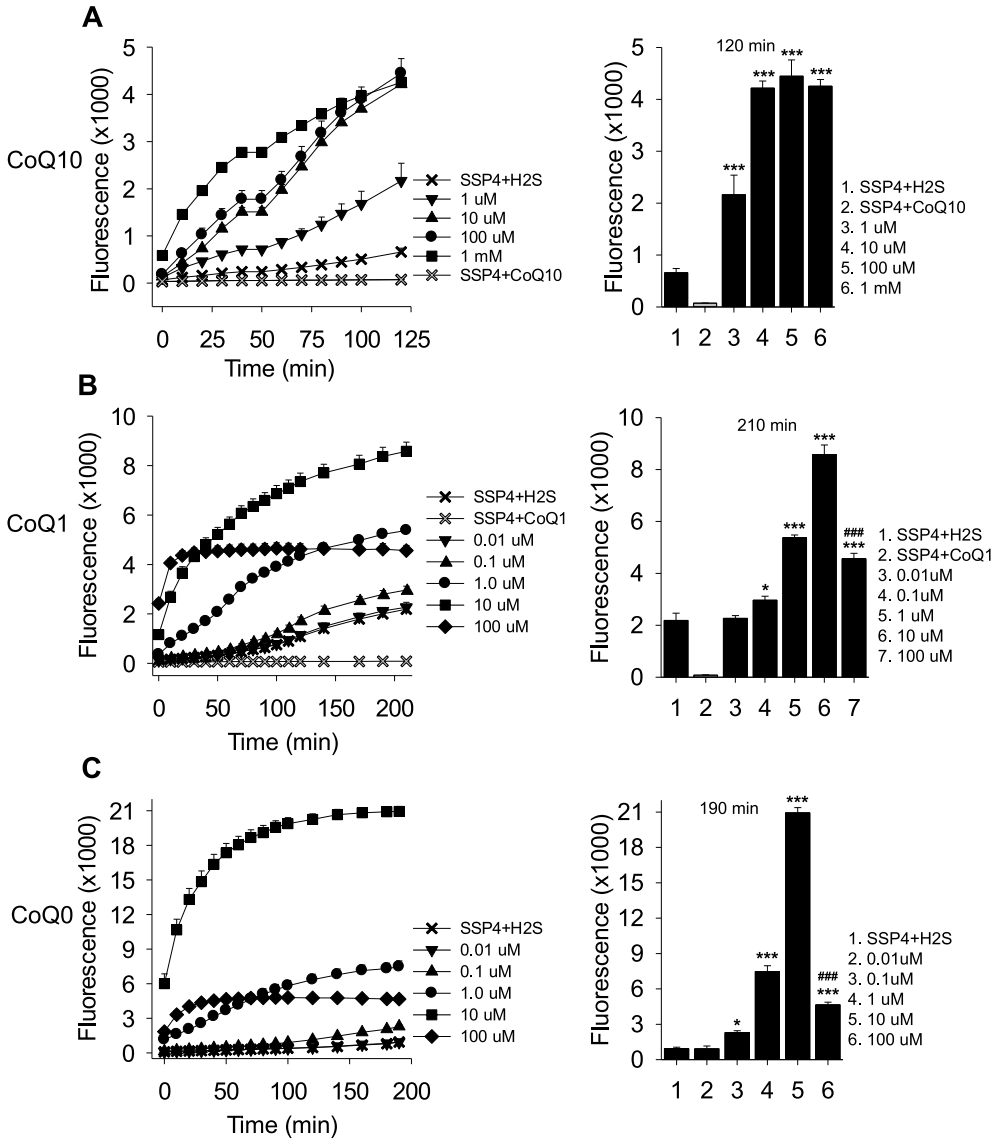

Fig. 2. CoQ_{10} (**A**), CoQ_1 (**B**) and CoQ_0 (**C**) concentration-dependently increased polysulfide production (5 μM SSP4 fluorescence) from 300 μM H_2S. Note different ordinate scales. Bar graphs show terminal values. Mean + SE, n = 4 wells per treatment; *, $p<0.05$, ***, $p<0.001$; significantly different from control (H_2S + SSP4); ###, $p<0.001$, significantly different from 10 μM CoQ_1 or CoQ_0; error bars may be hidden by symbols.

absence of CoQ_0, most likely due to volatilization and this was increased by CoQ_0. A small amount of H_2S_2 persulfide was also present either from autoxidation or as a contaminant in the H_2S solution. These results show that CoQ_0 rapidly oxidizes H_2S to per- and polysulfides.

Thiosulfate is another product of H_2S oxidation by quinones [10] and it is evident that CoQ_0 also oxidizes H_2S to thiosulfate (Fig. 3E and F). Approximately 10 μM of thiosulfate was either present as a contaminant or autoxidized to thiosulfate from 300 μM H_2S and this was further, and concentration-dependently, increased by CoQ_0 between 1 and 100 μM but then decreased by 300 μM CoQ_0. Around 40% of the initial 300 μM H_2S was converted to thiosulfate by 100 μM CoQ_0. These results show that thiosulfate is another significant product of H_2S oxidation by CoQ_0. H_2S may be oxidized to sulfite in culture medium which exerts additional cytoprotective effects [16]. While we did not measure sulfite in these experiments, we have previously shown that polyphenolic catechins in green tea oxidize H_2S to sulfite [8] suggesting that sulfite may also be a product of H_2S oxidation by CoQ compounds.

3.3. *H_2S oxidation by CoQ_1 and CoQ_0 is oxygen dependent, variously affected by superoxide dismutase and a catalytic process*

We have shown that hydroquinone must be oxidized to enable it to then oxidize H_2S, that this requires O_2 [10]. This suggests that H_2S oxidation of CoQ is also O_2 dependent. To verify that $CoQ_1$and CoQ_0 oxidation of H_2S are similar processes, we compared polysulfide formation by these quinones and H_2S in buffer equilibrated with room air (21% O_2) or sparged with 100% N_2 (0% O_2). Fig. 4 shows that H_2S oxidation is indeed O_2-dependent as polysulfide production in 21% O_2 was around three-fold greater than that in 0% O_2 at all CoQ_1 and CoQ_0 concentrations. The 0% O_2 samples could not be kept completely anaerobic and thus there was some production of polysulfides under these conditions.

We have also shown that the amount of polysulfide produced from H_2S by autoxidized hydroquinone is increased by superoxide dismutase (SOD) suggesting that H_2S is oxidized by the semiquinone rather than by superoxide and that the removal of superoxide helps drive the reaction

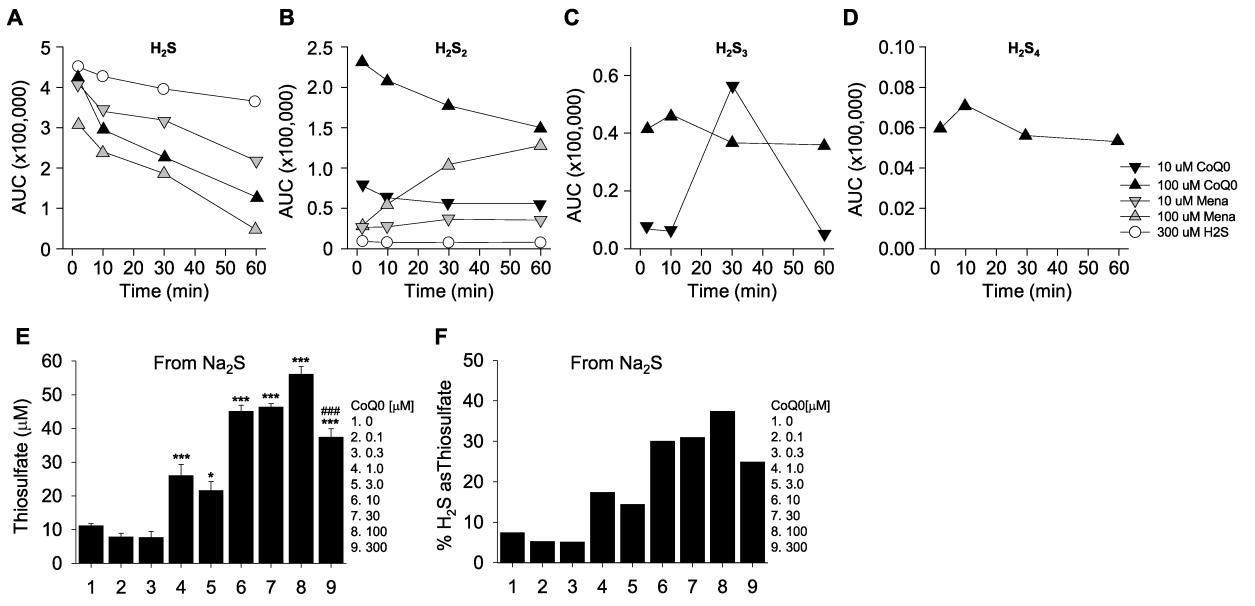

Fig. 3. Products of H_2S oxidation by CoQ_0 and menadione (Mena). (**A-D**) Typical experiment showing mass spectrometric analysis of polysulfide production from 300 μM H_2S catalyzed by 10 or 100 μM CoQ_0 or menadione (Mena) and derivatized with iodoacetamide at 2, 10, 30 and 60 min H_2S decreased faster in the presence of either CoQ_0 or Mena compared to H_2S alone. Polysulfides ($H_2S_{2\text{-}4}$) were rapidly produced by CoQ_0 whereas only H_2S_2 was produced by Mena and this slowly increased over time. A small amount of H_2S_2 was also present in H_2S but it did not change over time. Panels show the relative area under the curve (AUC) from extracted ion chromatograms. (**E-F**) Effects of CoQ_0 on thiosulfate production from 300 μM H_2S (as Na_2S). CoQ_0 from 1 to 100 μM concentration dependently increased thiosulfate production from H_2S. Values expressed as μM thiosulfate (**E**) or percent of H_2S converted to thiosulfate (**F**); both graphs corrected for thiosulfate produced by H_2S alone. Mean + SE, n = 4 wells per treatment; error bars may be hidden by symbols. *, ***, significantly different from 0 μM CoQ_0 at ($p<0.05$) and ($p<0.001$), respectively; ###, significantly different from 30 to 100 μM CoQ_0 at ($p<0.5$) and ($p<0.001$), respectively.

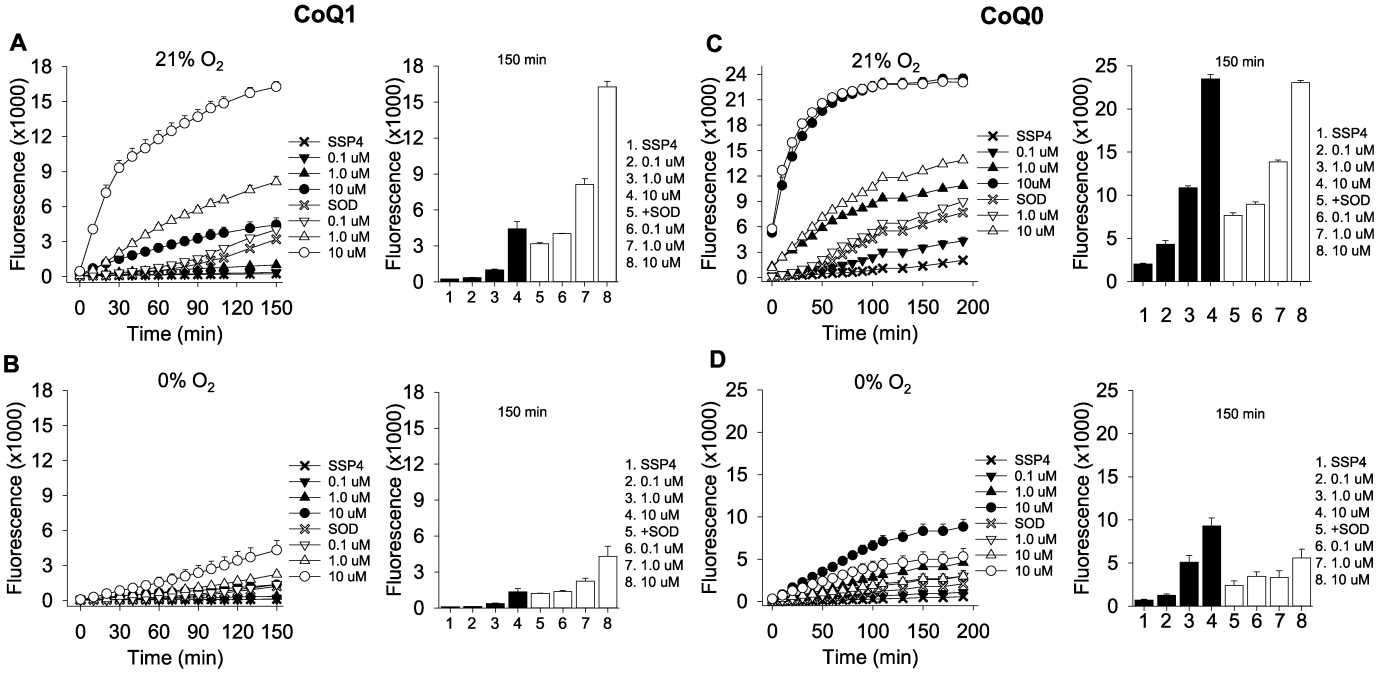

Fig. 4. Effects of oxygen and 0.1 μM superoxide dismutase on polysulfide production (5 μM SSP4 fluorescence) from CoQ_1 (**A**, **B**) and CoQ_0 (**C**, **D**) catalyzed oxidation of 300 μM H_2S; bar graphs show values at 150 min). (**A**) In 21% O_2 CoQ_1 concentration-dependently increased polysulfide formation and this was further increased by SOD at each CoQ_1 concentration ($p<0.001$). CoQ_0 also concentration-dependently increased polysulfide formation and this was significantly increased by SOD ($p<0.001$) at each CoQ_0 except 10 μM CoQ_0. In 0% O_2, CoQ_1 also concentration-dependently increased polysulfide formation and this was again significantly increased by SOD ($p<0.001$) at each CoQ_1 concentration. At 0% O_2, the effects of 0.1 and 1.0 μM CoQ_0 were not affected by SOD, whereas the effects of 10 μM CoQ_0 were significantly ($p = 0.014$) decreased by SOD. All CoQ_1 and CoQ_0 values are significantly ($p<0.001$) lower at 0% O_2 than at 21% O_2. Mean + SE, n = 4 wells per treatment; error bars may be hidden by symbols.

to the right [10]. Superoxide may also oxidize polysulfides [17]. As shown in Fig. 4, SOD increased polysulfide formation by CoQ_1 in both 0% and 21% O_2, albeit less dramatically in 0% O_2. SOD also increased polysulfide formation by 0.1 and 1 μM CoQ_0 in 21% O_2 but in 0% O_2 the only change was a slight, but significant ($p = 0.014$) decrease in polysulfide production by 10 μM CoQ_0. However, we have shown that SOD can directly oxidize H_2S to polysulfides [18] and this is again evident when comparing bars 1 (H_2S and SSP4) and 5 (H_2S and SSP4 with SOD) in all bar graphs in Fig. 4. If the effects of SOD alone are subtracted from CoQ_1 or CoQ_0 with SOD, SOD would still appear to have a stimulatory effect on CoQ_1 oxidation but an inhibitory effect on CoQ_0 at both O_2 tensions. Collectively, our results show that oxidation of H_2S by both CoQ_1 and CoQ_0 is O_2-dependent. The effects of SOD are less apparent, but because there is no consistent effect, the results suggest that H_2S is not oxidized by superoxide.

To further examine oxygen consumption by CoQ_0 oxidation of H_2S, we incubated various concentrations of CoQ_0 and H_2S while continuously monitoring oxygen tension (Fig. 5A–D). CoQ_0 concentration-dependently increased the rate of O_2 consumption with 300 μM H_2S; nearly all O_2 was consumed with 50 μM CoQ_0 plus 1 mM H_2S. Reversing the order of addition did not change the pattern of O_2 consumption (not shown). In these experiments addition of CoQ_0 produced a brief linear decrease in O_2 concentration followed by a longer exponential decrease. Under these conditions, O_2 consumption appeared to reach a nadir around 50% of room air, i.e., 12.5% O_2, but it would resume after addition of a second and third bolus of 300 μM H_2S (Fig. 5E). Three consecutive additions of CoQ_0 after H_2S produced similar results but the amount of O_2 consumed was less and appeared to become asymptotic (Fig. 5F). O_2 consumption was unaffected by either H_2S or CoQ_0 alone (Fig. 5G and H).

The catalytic properties of CoQ_0-mediated H_2S oxidation were further confirmed by measuring H_2S concentration in buffer with an

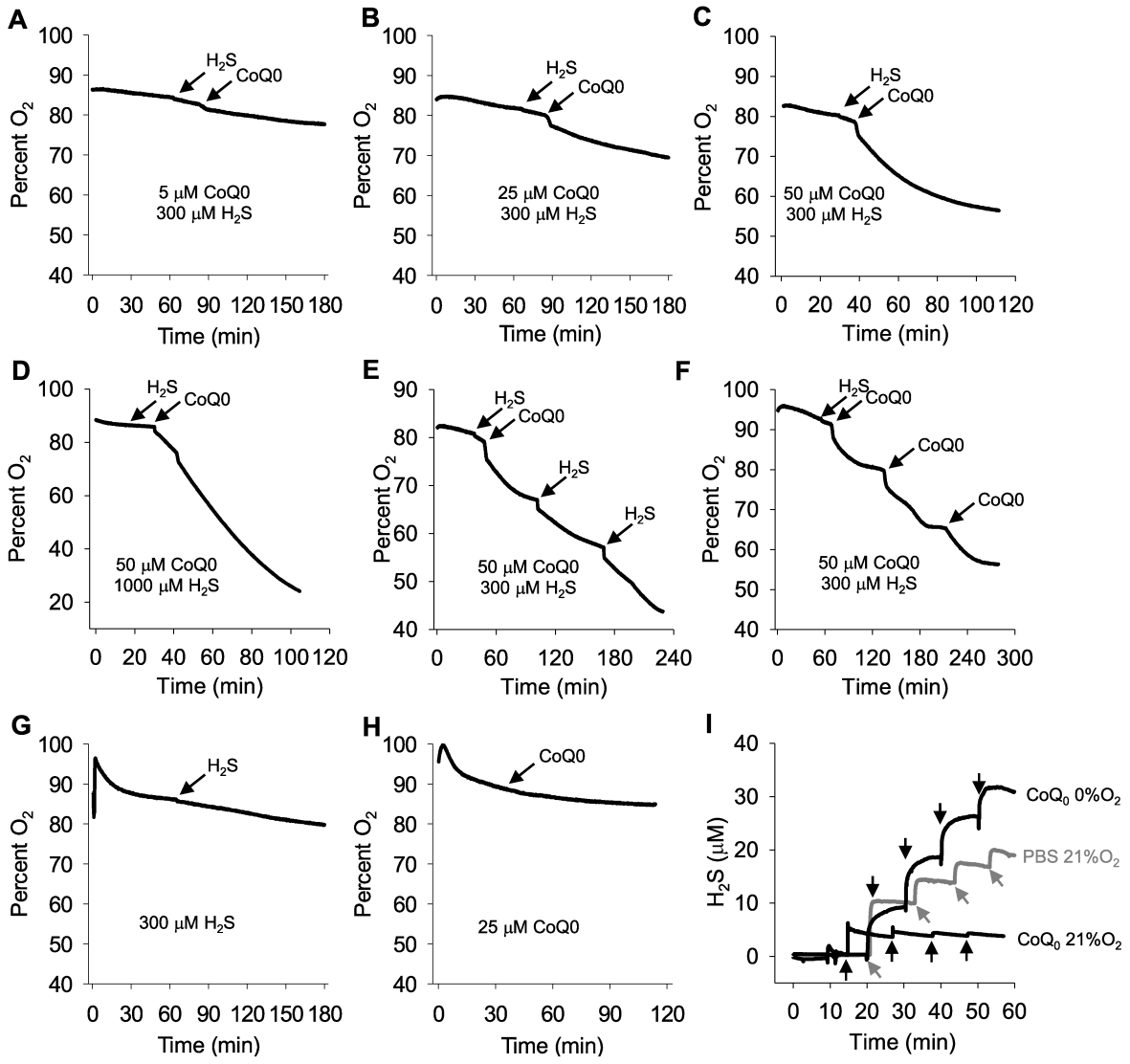

Fig. 5. Oxidation of H_2S by CoQ_0 consumes O_2 and it is dependent on CoQ_0 concentration. O_2 consumption was continuously monitored with a non-oxygen consuming O_2 sensor during additions of various concentrations of H_2S and CoQ_0 (**A-D**; inset in panels). O_2 consumption was further increased by consecutive additions of either 300 μM H_2S to CoQ_0 (**E**) or 50 μM additions of CoQ_0 to H_2S (**F**). O_2 consumption was not appreciably affected by either H_2S (**G**) or CoQ_0 (**H**) alone. (**I**) H_2S consumption was continuously monitored with an amperometric H_2S sensor during four sequential additions of 10 μM of H_2S (arrows) in buffer at 21% O_2 and in the presence of 10 μM CoQ_0 at either 21% or 0% O_2. The effect of H_2S in buffer at 21% O2 was somewhat reduced compared to CoQ0 at 0% O_2 most likely due to slow H_2S oxidation in 21% O_2; H_2S was rapidly removed by CoQ0 in 21% O_2.

amperometric H_2S sensor during four sequential additions of 10 μM H_2S in the absence of CoQ_0 and in the presence of 10 μM CoQ_0 at 21% and 0% O_2. As shown in Fig. 5I, H_2S is rapidly consumed by CoQ_0 in 21% O_2 but not in 0% O_2 or in buffer alone. Collectively, the above results support for our hypothesis that H_2S oxidation by CoQ_0 is an O_2-consuming, catalytic process.

3.4. Absorbance spectra of CoQ is consistent with redox cycling of the quinone

For CoQ_0 to catalytically oxidize H_2S to antioxidant polysulfides, CoQ_0 must redox cycle between the oxidized ubiquinone and either the semiquinone (ubisemiquinone) or the reduced ubiquinol. Redox cycling of many para-quinones is associated with spectral shifts, typically becoming red-shifted as the quinone is reduced to the quinol and blue-shifted as the process is reversed. CoQ_0 has reported absorption peaks at 268 and 289 nm for the oxidized quinone and the quinol reduced by $NaBH_4$, respectively [19]. We observed a similar absorption spectrum for oxidized CoQ_0 in buffer and this was red shifted after reduction with $NaBH_4$ and restored after 10 min in 21% O_2 (Supplemental Fig. S2A). We also observed a small, broad peak at 404 nm which disappeared after addition of $NaBH_4$. The 410 nm peak is suggestive of a semiquinone, as described by Land et al. [20]. A number of studies were then conducted to further examine the spectrum of CoQ_0 during its reaction with H2S under various conditions.

The effects of H_2S on the absorbance spectrum of CoQ_0 at different H_2S:CoQ_0 concentration ratios in normoxic and hypoxic buffer (21% and 0% O_2) are shown in Fig. 6. In normoxia, addition of H_2S in a 1:1 M ratio with CoQ_0 (Fig. 6A) produced a transient decrease in the 268 nm peak that reached a nadir in approximately 350 s, the rate of decrease appeared to be slightly faster approximately 100 s after the start of the experiment. Absorbance at 268 nm returned toward the initial absorbance by 1500 s and leveled off but did not quite reach the absorbance observed at the start of the experiment. Absorbance of the 288 nm peak increased for the initial 350 s and plateaued. H_2S also transiently decreased the 410 peak with the same time course as the 268 peak. The H_2S absorbance spectrum (229 nm) decreased relatively slowly for the initial 150 s then rapidly decreased between 150 and 500 s before the rate of decrease slowed again in an apparent exponential decline.

The absorbance spectrum of a 1:1 H_2S:CoQ_0 concentration in hypoxia is shown in Fig. 6B. Unlike in normoxia, addition of H_2S in hypoxia decreased absorbance at 268 nm over the initial 600 s but the absorbance did not recover. H_2S also increased absorbance at 288 nm and decreased it at 410 nm, however, neither response was as great as that observed in normoxia. H_2S absorbance at 229 nm decreased rapidly from 0 to 200 s then slowed for 50 s before increasing again; it then leveled off at 100 s H_2S absorbance decreased approximately 0.15 absorbance units in normoxia but only a little more than 0.04 units in hypoxia.

Increasing the H_2S:CoQ_0 to 4:1 produced a rapid drop over 200 s in

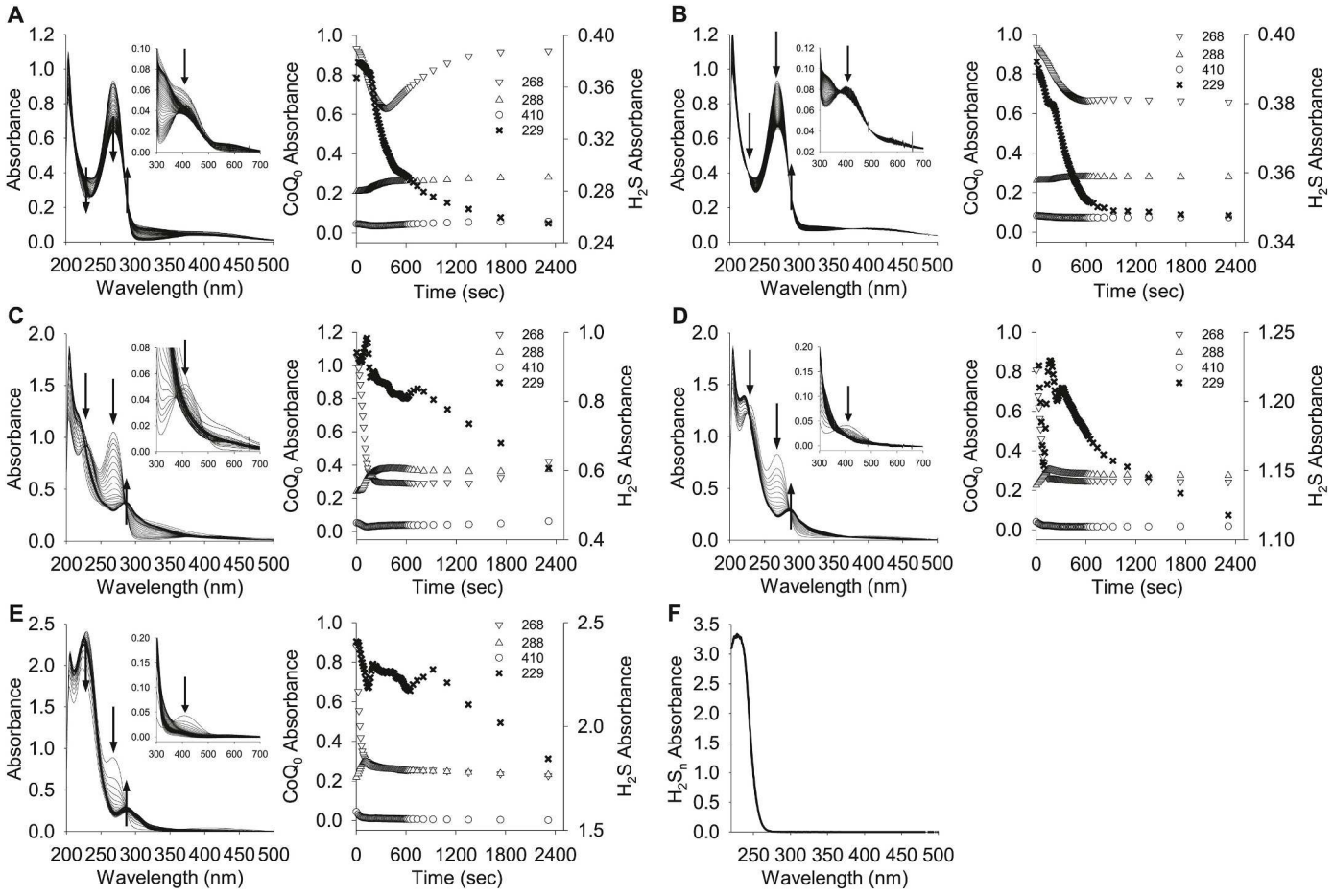

Fig. 6. Absorption spectra of H_2S oxidation by 454 μM CoQ_0 in 200 mM phosphate buffer (pH 7.4) in 21% O_2 (**A**, **C**, **E**) and 0% O_2 (**B, D**) at H_2S:CoQ_0 concentration ratios of 1:1 (**A**, **B**), 4:1 (**C, D**), or 10:1 (**E**). Left panels show absorbance as a function of wavelength (200–500 nm) for 1–2200 s, insets show expanded absorbance for 300–700 nm and right panels show absorbance of H_2S (229 nm), oxidized CoQ_0 (268 nm), reduced CoQ_0 (268 nm) and putative semiquinone (410 nm) as a function of time. At a 1:1 ratio, H_2S transiently reduced CoQ_0 in 21% O_2, produced a sustained reduction in 0% O_2, and there was a slight increase in oxidized CoQ_0. There was a more pronounced and sustained reduction of CoQ_0 at 4:1 and 10:1H_2S:CoQ_0 ratios. Absorbance of the putative semiquinone (410 nm) was decreased by H_2S with a more pronounced effect at higher H_2S:CoQ_0 ratios. (**F**) Absorbance of 1 mM H_2S (as Na_2S) in 100 mM phosphate buffer (pH 7.4) for reference.

absorbance of the 268 nm peak in both normoxia and hypoxia (Fig. 6C and 6D, respectively). Absorbance started to increase after 1200 s in normoxia but showed no signs of recovery in hypoxia. The decrease in the 268 nm peak coincided with an increase in 288 nm absorbance in both normoxia and hypoxia and the effects were more pronounced at the higher H_2S ratios. Absorbance of the 288 nm peak in normoxia appeared to plateau asymptotically over 600 s whereas absorbance of the hypoxic sample increased linearly for 150 s and then abruptly started to decrease. The decrease in absorbance of the 420 nm peak was also slightly more pronounced with the 4:1 H_2S:CoQ_0 concentration ratio. The effects on the H_2S absorbance at 229 nm were erratic but showed a general decline after 300–600 s. The effects of a 10:1 H_2S:CoQ_0 concentration ratio in normoxia were similar to those of a 4:1 ratio, albeit with slightly faster responses and the shape of the 288 nm curve was more like that of the hypoxic 4:1 curve.

These results suggest that CoQ_0 readily redox cycles and that H_2S oxidation by CoQ_0 is also reversible and depends on the relative concentrations of both H_2S and O_2. To examine this further, we first reduced CoQ_0 with H_2S and then bubbled the mixture with nitrogen. This slowly decreased the reduced CoQ_0 peak but there was no evidence that the CoQ_0 was reoxidized (Fig. 6B) until the mixture was then bubbled with air (Fig. 6C). We also confirmed that the H_2S absorbance peak was 229 nm (Fig. 6F).

Collectively, these results show that H_2S reduces CoQ_0 in a reaction that consumes H_2S and is enhanced by O_2 and that this reaction proceeds relatively quickly over the initial 10 min. Furthermore, with a 1:1 concentration ratio of H_2S:CoQ_0, our results suggest that the reduced CoQ_0 is readily reoxidized, presumably after sufficient H_2S is consumed. This supports our hypothesis that it is a catalytic process. The absorbance response of the 410 nm peak, which we suggest is attributable to H_2S oxidation of the semiquinone, also suggests that this molecule is intimately involved in redox cycling of CoQ_0. The erratic responses of the H_2S spectrum at high H_2S ratios may be complicated by other sulfur species, which is supported by the LCMS findings.

3.5. CoQ_0 depletes cellular H_2S and increases polysulfides and thiosulfate

To examine the effects of CoQ_0 on cellular metabolism we measured H_2S (AzMC fluorescence) and polysulfide (SSP4 fluorescence) in HEK293 cells at 5% and 21% O_2 over 22 h. Because the 5% O_2 cells had to be removed from the hypoxia chamber for measurements, they were only examined at 0, 60, 240 min and 22 h. Thiosulfate was also measured in the medium at the end of the experiment. The effects of CoQ_0 on H_2S and polysulfide metabolism were also examined in HT29 cells at 21% O_2.

As shown in Fig. 7A and B, CoQ_0 concentration-dependently decreased cellular H_2S and increased polysulfide in both 21% and 5% O_2. CoQ_0 from 1 to 10 μM also increased thiosulfate excretion into the medium. Higher concentrations of CoQ_0 (30 and 100 μM) appeared to decrease thiosulfate excretion into the medium. CoQ_0 had similar effects on H_2S and polysulfide metabolism in HT29 cells at 21% O_2 Fig. 7C); effects on thiosulfate production were not examined. These results show that the effects of CoQ_0 on sulfur metabolism in HEK293 and HT29 cells are consistent with its effects on H_2S in buffer. We attribute the decrease in H_2S to a CoQ_0-mediated oxidation of endogenous H_2S which is reflected by an increase in cellular production of both polysulfides and

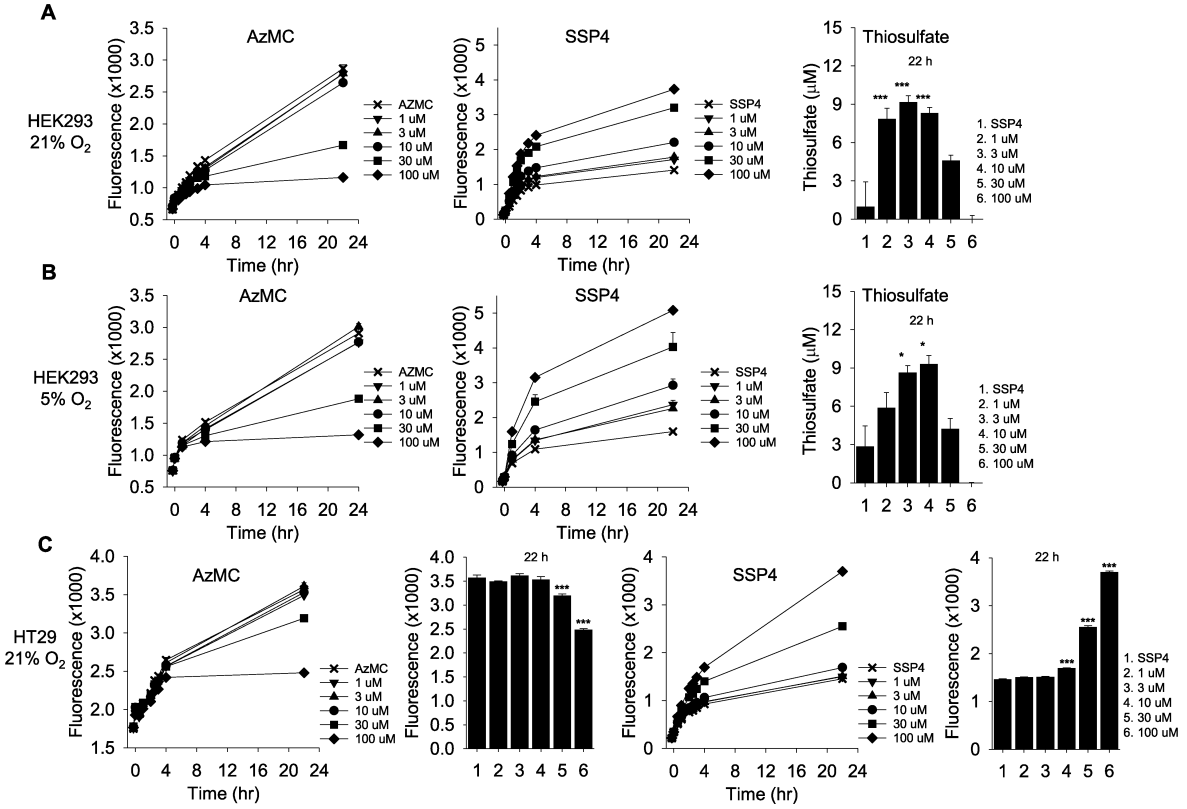

Fig. 7. Effects of CoQ_0 on H_2S (AzMC fluorescence; left, line graphs), polysulfides (10 μM SSP4 fluorescence; right, line graphs) and thiosulfate (bar graphs) in HEK293 cells exposed to 21% O_2 (**A**) and 5% O_2 (**B**). CoQ_0 concentration-dependently decreased cellular H_2S (25 μM AzMC fluorescence), increased polysulfide (10 μM SSP4 fluorescence) and at 3–10 μM increased thiosulfate excretion into the medium. Higher concentrations of CoQ_0 appeared to increase polysulfide production and decrease thiosulfate excretion at 0% O_2 compared to 21% O_2. (**C**) Similar effects of CoQ_0 on H_2S and polysulfide metabolism were observed in HT29 cells at 21% O_2. Mean + SE, n = 8 wells per treatment; error bars may be hidden by symbols.

thiosulfate in HEK293 cells and polysulfides in HT29 cells. The effects of O_2 are less clear as they could be attributed to direct autoxidation of CoQ_0 in either the medium or cells, or to effects on the ETC and overall sulfur metabolism.

3.6. H_2S oxidation by related quinones, MitoQ, menadione and idebenone

3.6.1. MitoQ

MitoQ, CoQ_{10} linked to triphenylphosphonium, is rapidly taken up by cells and accumulated in the mitochondria. The reduced quinol form of MitoQ is not oxidized by complex III, whereas, when oxidized, it is readily reduced by complex II, which gives it is main purported function as an antioxidant [21]. As expected, MitoQ also readily, and concentration-dependently, oxidized H_2S to polysulfides in buffer with an apparent efficacy between that of CoQ_0 and CoQ_1 (Supplemental Fig. S3A). However, unlike CoQ_0 or CoQ_1, SSP4 fluorescence did not decrease at the highest MitoQ concentrations. In HEK293 cells, 100 μM MitoQ slightly, but significantly decreased AzMC fluorescence and increased SSP4 fluorescence at 22 h (Supplemental Figs. S3B and C). Lower concentrations were ineffective. These results show that MitoQ readily oxidizes H_2S to polysulfides in buffer. MitoQ also appears to consume cellular H_2S to produce polysulfides in cells, although it is considerably less potent than CoQ_0.

3.6.2. Menadione

Menadione (Vitamin K_3) is a fat-soluble vitamin precursor similar to CoQ_0 but without the 2,3 methoxy groups. Menadione from 0 to 10–30 μM concentration-dependently oxidized H_2S to polysulfides and thiosulfate in buffer, whereas above these concentrations both polysulfide and thiosulfate production decreased (Supplemental Figs. S4A–C). Menadione also decreased H_2S and increased polysulfide production in both HEK293 and HT29 cells (Supplemental Figs. S4D and E).

3.6.3. Idebenone

Idebenone is a more water-soluble analog of CoQ_{10} with ten methylene molecules and a terminal hydroxyl replacing the isoprenyl groups of CoQ_{10} [22]. Idebenone concentration-dependently increased polysulfide production from H_2S in buffer and slightly, but significantly decreased H_2S and increased polysulfides in HEK293 cells exposed to 100 μM idebenone (Supplemental Fig. S5).

4. Discussion

CoQ_{10} is present in all eukaryote cells [23]. While CoQ_{10} is generally associated with mitochondrial membranes, it is found in higher concentrations in the golgi membranes [24] and it is also present in endoplasmic reticulum and plasma membranes. Its membrane confines are due to the lipophilic isoprenyl tail. CoQ_{10} readily redox cycles between the oxidized ubiquinone, a semiquinone (ubsemiquinone) intermediate and the fully reduced ubiquinol. It is best known as a mitochondrial oxidant for its ability to shuttle electrons down the electron transport chain (ETC) from complex I and II, but it also moves electrons from electron-transferring flavoprotein ubiquinone oxidoreductase (ETF-QOR), glycerol 3-phosphate dehydrogenase (G3PDH), dihydroorotate dehydrogenase (DHODH), choline dehydrogenase (CHDH), proline dehydrogenase (PRODH) as well as SQR [23].

Our results show that CoQ_{10}, its more truncated and hydrophilic derivatives, CoQ_1 and CoQ_0, and related quinones, MitoQ, menadone and idebenone, catalyze the oxidization of H_2S to polysulfides and thiosulfate in buffer independent of SQR or other enzymes. These reactions require oxygen and are augmented by addition of SOD. We also show that CoQ_0 decreases endogenous H_2S in both HEK293 and HT29 cells, increases intracellular polysulfides and increases thiosulfate excretion into the medium. These results suggest that CoQ_0 has similar effects on sulfur metabolism in cells as it does in buffer. MitoQ, menadione and idebenone similarly decrease cellular H_2S and increase polysulfides, although MitoQ and idebenone appear less efficacious. Collectively, these results suggest that H_2S can be metabolized in the absence of a functioning SQR-ETC system either in the mitochondrion or other membranes where ubiquinone is present, or by addition of various CoQ and CoQ-like molecules. The decreased efficacy of mitoQ in affecting cellular sulfur metabolism may be due to its localization in the mitochondrion, while most of the H_2S production is cytosolic. Clearly, more specific studies on the effects of MitoQ on intra-mitochondrial sulfur metabolism are needed, as well as identification of intracellular distribution of idebenone to resolve these issues.

4.1. Mechanism of action

We previously reported that benzoquinone (BQ), or hydroquinone (HQ) that was autoxidized by oxygen, readily oxidizes H_2S in buffer and cells and produces polysulfide and thiosulfate [10]. Those results are similar to the effects of the quinones used herein and they suggest that these reactions involve oxygen-dependent redox cycling of the quinone. While a detailed examination of H_2S oxidation by quinones was beyond the scope of either study, there are a number, albeit not comprehensive, of one- and two-electron reactions that deserve mention. These are described below and summarized in Fig. 8. It should also be noted that the products of these reactions may react with each other to produce numerous per-polysulfides and sulfoxides, only a few of which are described here. Note: the pKa of polysulfides decreases as the number of sulfur atoms increases and most polysulfides are anions or dianions [25]. For convenience they are represented here as the fully protonated form.

One-electron reactions likely involve the initial formation of a semiquinone radical (Fig. 8A). In this reaction, oxygen autoxidizes a hydroquinone (QH_2) producing a semiquinone radical ($Q^{\bullet}$) and superoxide ($O_2^{\bullet-}$; eq. (1)):

$$QH_2 + O_2 \rightarrow Q^{\bullet} + O_2^{\bullet-} + 2H^+ \quad (1)$$

The semiquinone, $Q^{\bullet}$ could then oxidize H_2S (or more likely the hydrosulfide anion, HS^-) to a hydrosulfide radical ($HS^{\bullet}$) regenerating the reduced quinone, QH_2 (eq. (2));

$$HS^- + Q^{\bullet} + 2H^+ \rightarrow HS^{\bullet} + QH_2 \quad (2)$$

Alternatively, the semiquinone could undergo a second one-electron oxidation to a quinone using either superoxide or oxygen, producing either hydrogen peroxide or superoxide, respectively, and then the quinone oxidizes a second hydrosulfide anion (eqs. (3) and (4));

$$Q^{\bullet} + O_2^{\bullet-}\ (O_2) + 2H^+ \rightarrow Q + H_2O_2\ (O_2^{\bullet-}) \quad (3)$$

$$HS^- + Q \rightarrow HS^{\bullet} + Q^{\bullet} \quad (4)$$

In a two-electron reaction the hydroquinone would be directly oxidized by oxygen to the quinone while producing hydrogen peroxide (eq. (5));

$$QH_2 + O_2 \rightarrow Q + H_2O_2 \quad (5)$$

and the quinone would then oxidize hydrosulfide, also via a two-electron step, to elemental sulfur (S^0; eq. (6));

$$HS^- + Q \rightarrow S^0 + QH_2 \quad (6)$$

It is also possible that either reaction (eq. (5) or 6) consists of two sequential one-electron reactions while the other is a single two-electron process.

The above schemes are further complicated by comproportionation of a quinone and hydroquinone to two semiquinones (eq. (7)) which further sustain the above redox reactions;

$$QH_2 + Q \rightarrow 2Q^{\bullet} \quad (7)$$

Two hydrosulfide radicals produced in reactions 2 and 4 may then

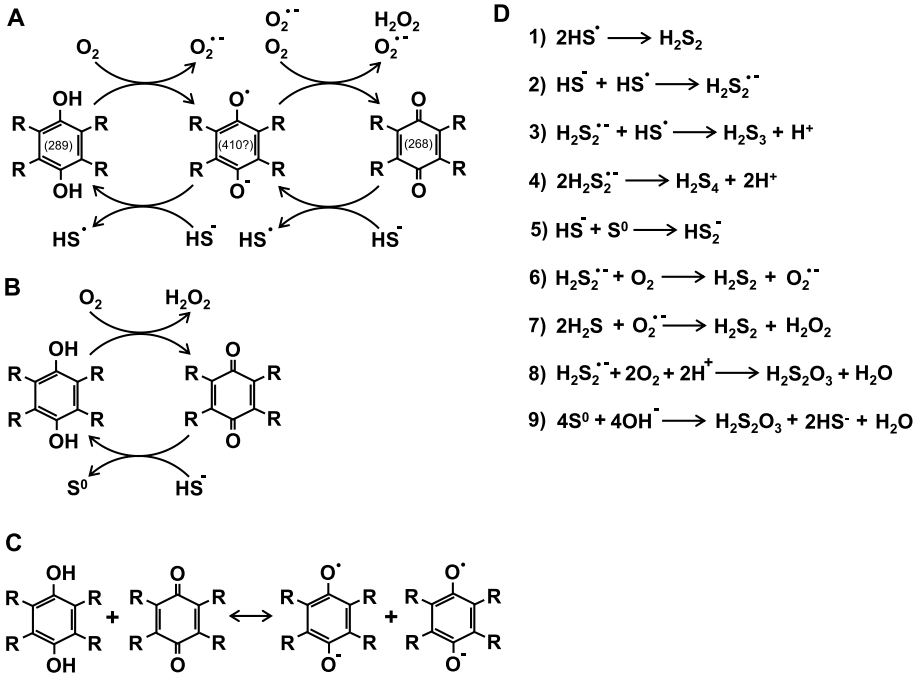

Fig. 8. Potential reactions of H_2S oxidation by quinones; R indicates various quinone side groups, absorbance peaks (nm) are shown in parentheses. **A**) Left to right: oxygen-dependent sequential one-electron oxidations of a hydroquinone initially produce a semiquinone radical and superoxide (O_2,.). In the second step the semiquinone is oxidized to a quinone by either superoxide to produce hydrogen peroxide (H_2O_2) or oxygen to produce superoxide. Hydrosulfide anions (HS^-) are oxidized to hydrosulfide radicals ($HS^•$) in the reverse reactions. **B**) Oxygen-dependent two-electron oxidation of a hydroquinone produces a quinone and peroxide and hydrosulfide anions are oxidized to elemental sulfur (S^0) in the reverse reaction. (**C**) Possible reactions following H_2S oxidation. See text for further details.

combine to form a persulfide (H_2S_2; eq. (8));

$$2HS^• \text{ –> } H_2S_2 \quad 8$$

or one thiyl radical could react with one hydrosulfide ion to produce a dihydrodisfulfide (persulfide) radical (eq. (9));

$$HS^• + HS^- \text{ –> } H_2S_2^{•-} \quad 9$$

The persulfide radical reaction with O_2 will produce a persulfide and superoxide (eq. (10));

$$H_2S_2^{•-} + O_2 \text{ –> } H_2S_2 + O_2^{•-} \quad 10$$

The hydrosdulfide radical can also react with the persulfide radical to produce a trisulfide (eq. (11));

$$HS^• + H_2S_2^{•-} \text{ –> } H_2S_3 + H^+ \quad 11$$

or two persulfide radicals can combine to form a tetrapolysulfide (eq. (12));

$$2H_2S_2^{•-} \text{ –> } H_2S_4 + 2H^+ \quad 12$$

A persulfide could also be formed by the reaction between hydrosulfide and elemental sulfur (eq. (13));

$$HS^- + S^0 \text{ –> } H_2S_2 \quad 13$$

However, we propose that a two-step reaction (eqs. 1 and 3) is involved in H_2S oxidation by quinones. First, our spectral studies show that CoQ_0 is fully reduced by H_2S (Fig. 4) suggesting this as the starting point for subsequent oxidative steps of the hydroquinone. Second, we show that SOD increases polysulfide production (Fig. 6). This would not be expected if H_2S was oxidized to elemental sulfur in a single 2-electron reaction starting with the fully oxidized quinone (eqs. (5) and (6)) as there would be no superoxide intermediate. Furthermore, SOD has been shown to increase oxidation of a semiquinone to a quinone [26]. We showed that SOD also increased polysulfide formation (Fig. 4) suggesting that the quinone is a more efficient starting point for H_2S oxidation than a semiquinone.

The above reactions can also produce a variety of sulfoxides that, with the exception of thiosulfate, were not examined in this study. Thiosulfate could be produced by a reaction between the persulfide and superoxide (eq. (14));

$$H_2S_2 + 2O_2^{•-} \text{ –> } S_2O_3^{2-} + H_2O \quad 14$$

between a persulfide radical and superoxide (eq. (15));

$$H_2S_2^{•-} + 2O_2^{•-} \text{ –> } O_2 + S_2O_3^{2-} + H_2O \quad 15$$

or even starting with molecular sulfur (eq. (16))

$$4S^0 + 4OH^- \text{ –> } S_2O_3^{2-} + 2HS^- + H_2O \quad 16$$

4.2. *Potential functions of ubiquinone and related quinones in sulfur metabolism*

We propose that direct H_2S metabolism by ubiquinone, independent of the ETC, may serve several functions including 1) formation signaling persulfides, 2) production of thiosulfate, 3) function as antioxidant, 4) provide a therapeutic option to affect cellular H_2S. These hypotheses are briefly discussed below but they clearly await experimental confirmation.

4.2.1. *Formation signaling persulfides*

The function of polysulfides as mediators of H_2S signaling through persulfidation of thiols on regulatory proteins is well established and the reader is referred to a number of recent reviews [3,27–31]. The ability of quinones to form polysulfides at various locations within the cell, especially if there is concomitant H_2S formation nearby is an attractive possibility for generating signaling polysulfides within specific intracellular domains.

4.2.2. *Production of thiosulfate*

Thiosulfate is an intermediate in the mitochondrial pathway of H_2S oxidation to sulfate for ultimate excretion [3]. It is also a source of H_2S [32,33] and there is accumulating evidence that exogenous thiosulfate is protective in a variety of pathological situations [34–37]. It remains to be determined if increased endogenous thiosulfate production will have similar attributes.

4.2.3. Antioxidant functions

H_2S/ubiquinone redox cycling can have two different antioxidant functions either by reducing ubiquinone to ubiquinol or by production of polysulfides. In the mitochondrion, CoQ_{10} prevents electrons from leaking out of the ETC and reducing oxygen to superoxide and it binds to common sites involved with the mitochondrial permeability transition pore (MPTP), preventing pore formation and membrane depolarization [38]. Extra-mitochondrial CoQ_{10} protects the golgi apparatus from ROS [24] and in plasma membranes CoQ_{10} inhibits ferroptosis as a lipophilic radical-trapping antioxidant that halts the propagation of lipid peroxides [39].

Polysulfides are potent direct antioxidants [28], they also scavenge ROS, especially superoxide [17], and they persulfidate Keap1 which frees it from Nrf2 allowing the latter to activate the genomic antioxidant response elements [40–45]. Clearly, polysulfides formed from CoQ_{10} oxidation of H_2S could serve a variety of antioxidant functions within in the mitochondrion and at specific extra-mitochondrial locations throughout the cell. However, it should be noted that this may be a double-edged sword as is also possible that the additional oxidants are produced by this reaction (superoxide and hydrogen peroxide) may have detrimental effects.

4.2.4. An alternative H_2S oxidase and/or detoxification pathway

SQR is regarded as the primary pathway for H_2S catabolism in vertebrates. It is proton-motive as it feeds electrons via ubiquinone into the electron transport chain where they are delivered to complex III. SQR is present in most mammalian cells, especially colonic epithelia and HT29 cells, but it appears to be noticeably lacking in the CNS and in HEK293 cells [6,46]. This suggests that neurons and HEK293 cells possess alternative mechanisms for H_2S metabolism.

Alternative oxidases allow for the transfer of electrons to oxygen without concomitant proton transport and ATP synthesis. They were originally thought to occur only in plants but have since been identified in several animal phyla [47]. Invertebrates inhabiting sulfidic environments have such a pathway that is used to metabolize H_2S [48,49], but its existence in vertebrates is less clear. Our studies suggest that ubiquinone could function as an alternative oxidase for H_2S catabolism by directly transferring electrons from H_2S to O_2. In this way H_2S could still be oxidized when there is a strong proton gradient and when ATP stores are full. It would also function in cells where SQR is lacking or insufficient to keep up with H_2S production and it would be operative in non-mitochondrial membranes that are distant from the mitochondria. We show that both CoQ_0 and CoQ_1 decrease intracellular H_2S concentration and increase polysulfide concentration in these cells suggesting that they promote oxidation of endogenous H_2S. The broad distribution of CoQ_{10} in cellular membranes may also provide protection from elevated exogenous H_2S.

While it is difficult to separate the effects of SQR from CoQ_{10} on H_2S oxidation in most eukaryote cells, there are a number of studies that point to a specific role of CoQ_{10} in sulfide metabolism. The fission yeast, *Schizosaccharomyces pombe*, lacks SQR yet efficiently metabolizes H_2S. However, inhibition of CoQ_{10} biosynthesis increases H_2S production by *S. pombe* cells and the authors conclude that CoQ_{10} directly metabolizes H_2S [50].

4.3. Can sulfur metabolism explain biological actions of exogenous quinones?

The biological effects of idebenone (reviewed in [22]; and briefly summarized below) may offer a clue to its relatively poorly understood mechanism of action. Like CoQ_{10}, idebenone may undergo 1 or 2 electron redox reactions with a semiquinone intermediate. Idebenone was originally thought to serve as an effective antioxidant and to assist in electron transport, however, more recent studies suggest that this is not the case. Whole-animal studies have shown that cellular uptake of idebenone is relatively low, resulting in low nanomolar concentrations that are relatively quickly cleared from cells. This would suggest that idebenone does not function as an effective antioxidant or does it persist in cells long enough to contribute to electron transport in the mitochondrion. OS-10, a very biologically active idebenone metabolite, is even more water soluble than idebenone and likely exerts its actions in the cytosol because it does not enter mitochondria. In many instances, the benefits of idebenone are only realized in hypoxic situations, which is relevant to sulfur metabolism as cellular H_2S is increased in hypoxia [51]. Furthermore, recent work suggests that much of the benefit from idebenone results from activation of downstream effectors such as Nrf2 and AKT. These observations are consistent with idebenone oxidizing H_2S to polysulfides as the latter are well-known activators of both Nrf2 [40–42,52–57].

4.4. Clinical relevancy of H_2S oxidation by CoQ_{10}

H_2S has long been known as a toxic gas, however, there is accumulating evidence for its cytoprotective roles. While much of the toxicity results from H_2S inhibition of complex IV (cytochrome C oxidase; CCO) many of the benefits accrue from its support of mitochondrial function as summarized in a recent review [58]. The difference between these two outcomes is dependent on the concentration of H_2S which is affected by both the rate of H_2S production and consumption. Recent evidence suggests that increasing H_2S consumption by increasing expression of SQR, or by removing H_2S with small-molecule sulfonyl azide H_2S scavengers may be beneficial in instances of H_2S toxicity [59] or central nervous system ischemia, where hypoxia inhibits normal H_2S catabolism [60]. H_2S levels are also elevated in individuals with Down syndrome due to increased expression of chromosome 21-coded CSE [61]. We propose that derivatives of CoQ_{10} will provide an effective alternative therapeutic approach to decrease H_2S in conditions associated with its excess.

Declaration of competing interest

TAK is founder and stockholder in Gerenox, Inc. TAK, PJD and KRO have submitted for patent protection on Down Syndrome therapeutics. The other authors have no conflict of interest.

Acknowledgments

This research was supported in part by National Science Foundation Grant NO. IOS2012106(KRO), National institute of Health Grant NO R01NS094535 (TAK, PJD), Welch Foundation Grant BE-0048 (TAK) and Biomedical Research Foundation at Central Arkansas Veteran's Healthcare System (KDS). The authors gratefully acknowledge Dr. Mijoon Lee and Ms. Nonka Sevova at the University of Notre Dame Mass Spectrometry and Proteomics Facility for excellent technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.freeradbiomed.2022.02.018.

References

[1] K. Abe, H. Kimura, The possible role of hydrogen sulfide as an endogenous neuromodulator, J. Neurosci. 16 (1996) 1066–1071.
[2] J.B. Kohl, A.T. Mellis, G. Schwarz, Homeostatic impact of sulfite and hydrogen sulfide on cysteine catabolism, Br. J. Pharmacol. 176 (2019) 554–570.
[3] K.R. Olson, H2S and polysulfide metabolism: conventional and unconventional pathways, Biochem. Pharmacol. 149 (2018) 77–90.
[4] M.R. Jackson, P.J. Loll, M.S. Jorns, X-ray structure of human sulfide:quinone oxidoreductase: insights into the mechanism of mitochondrial hydrogen sulfide oxidation, Structure 27 (2019) 794–805 e794.
[5] M. Goubern, M. Andriamihaja, T. Nubel, F. Blachier, F. Bouillaud, Sulfide, the first inorganic substrate for human cells, Faseb. J. 21 (2007) 1699–1706.
[6] E. Lagoutte, S. Mimoun, M. Andriamihaja, C. Chaumontet, F. Blachier, F. Bouillaud, Oxidation of hydrogen sulfide remains a priority in mammalian cells

and causes reverse electron transfer in colonocytes, Biochim. Biophys. Acta 1797 (2010) 1500–1511.
[7] A.P. Landry, D.P. Ballou, R. Banerjee, H2S oxidation by nanodisc-embedded human sulfide quinone oxidoreductase, J. Biol. Chem. 292 (2017) 11641–11649.
[8] K.R. Olson, A. Briggs, M. Devireddy, N.A. Iovino, N.C. Skora, J. Whelan, B.P. Villa, X. Yuan, V. Mannam, S. Howard, Y. Gao, M. Minnion, M. Feelisch, Green tea polyphenolic antioxidants oxidize hydrogen sulfide to thiosulfate and polysulfides: a possible new mechanism underpinning their biological action, Redox Biol. 37 (2020), 101731.
[9] K.R. Olson, Y. Gao, A. Briggs, M. Devireddy, N.A. Iovino, M. Licursi, N.C. Skora, J. Whelan, B.P. Villa, K.D. Straub, 'Antioxidant' berries, anthocyanins, resveratrol and rosmarinic acid oxidize hydrogen sulfide to polysulfides and thiosulfate: a novel mechanism underlying their biological actions, Free Radic. Biol. Med. 165 (2021) 67–78.
[10] K.R. Olson, Y. Gao, K.D. Straub, Oxidation of hydrogen sulfide by quinones: how polyphenols initiate their cytoprotective effects, Int. J. Mol. Sci. 22 (2021).
[11] S.I. Bibli, B. Luck, S. Zukunft, J. Wittig, W. Chen, M. Xian, A. Papapetropoulos, J. Hu, I. Fleming, A selective and sensitive method for quantification of endogenous polysulfide production in biological samples, Redox Biol. 18 (2018) 295–304.
[12] K.R. Olson, Y. Gao, Effects of inhibiting antioxidant pathways on cellular hydrogen sulfide and polysulfide metabolism, Free Radic. Biol. Med. 135 (2019) 1–14.
[13] K.R. Olson, Y. Gao, F. Arif, S. Patel, X. Yuan, V. Mannam, S. Howard, I. Batinic-Haberle, J. Fukuto, M. Minnion, M. Feelisch, K.D. Straub, Manganese porphyrin-based SOD mimetics produce polysulfides from hydrogen sulfide, Antioxidants (2019) 8.
[14] C. Dong, Z. Wang, Y. Zhang, X. Ma, M.Z. Iqbal, L. Miao, Z. Zhou, Z. Shen, A. Wu, High-performance colorimetric detection of thiosulfate by using silver nanoparticles for smartphone-based analysis, ACS Sens. 2 (2017) 1152–1159.
[15] P.M. May, D. Batka, G. Hefter, E. Konigsberger, D. Rowland, Goodbye to S(2-) in aqueous solution, Chem. Commun. 54 (2018) 1980–1983.
[16] Y. Kimura, N. Shibuya, H. Kimura, Sulfite protects neurons from oxidative stress, Br. J. Pharmacol. 176 (2019) 571–582.
[17] R. Wedmann, S. Bertlein, I. Macinkovic, S. Boltz, J. Miljkovic, L.E. Munoz, M. Herrmann, M.R. Filipovic, Working with "H2S": facts and apparent artifacts, Nitric Oxide 41 (2014) 85–96.
[18] K.R. Olson, Y. Gao, F. Arif, K. Arora, S. Patel, E.R. DeLeon, T.R. Sutton, M. Feelisch, M.M. Cortese-Krott, K.D. Straub, Metabolism of hydrogen sulfide (H2S) and production of reactive sulfur species (RSS) by superoxide dismutase, Redox Biol. 15 (2017) 74–85.
[19] T. Takahashi, Y. Mine, T. Okamoto, 2,3-Dimethoxy-5-methyl-p-benzoquinone (coenzyme Q0) disrupts carbohydrate metabolism of HeLa cells by adduct formation with intracellular free sulfhydryl-groups, and induces ATP depletion and necrosis, Biol. Pharm. Bull. 41 (2018) 1809–1817.
[20] E.J. Land, M. Simic, A.J. Swallow, Optical absorption spectrum of half-reduced ubiquinone, Biochim. Biophys. Acta 226 (1971) 239–240.
[21] R.A. Smith, M.P. Murphy, Animal and human studies with the mitochondria-targeted antioxidant MitoQ, Ann. N. Y. Acad. Sci. 1201 (2010) 96–103.
[22] N. Gueven, P. Ravishankar, R. Eri, E. Rybalka, Idebenone: when an antioxidant is not an antioxidant, Redox Biol. 38 (2021), 101812.
[23] Y. Wang, S. Hekimi, Understanding ubiquinone, Trends Cell Biol. 26 (2016) 367–378.
[24] V. Mugoni, R. Postel, V. Catanzaro, E. De Luca, E. Turco, G. Digilio, L. Silengo, M. P. Murphy, C. Medana, D.Y. Stainier, J. Bakkers, M.M. Santoro, Ubiad1 is an antioxidant enzyme that regulates eNOS activity by CoQ10 synthesis, Cell 152 (2013) 504–518.
[25] A. Kamyshny Jr., A. Goifman, J. Gun, D. Rizkov, O. Lev, Equilibrium distribution of polysulfide ions in aqueous solutions at 25 degrees C: a new approach for the study of polysulfides' equilibria, Environ. Sci. Technol. 38 (2004) 6633–6644.
[26] Y. Song, G.R. Buettner, Thermodynamic and kinetic considerations for the reaction of semiquinone radicals to form superoxide and hydrogen peroxide, Free Radic. Biol. Med. 49 (2010) 919–962.
[27] M.R. Filipovic, J. Zivanovic, B. Alvarez, R. Banerjee, Chemical biology of H2S signaling through persulfidation, Chem. Rev. 118 (2018) 1253–1337.
[28] J.M. Fukuto, L.J. Ignarro, P. Nagy, D.A. Wink, C.G. Kevil, M. Feelisch, M. M. Cortese-Krott, C.L. Bianco, Y. Kumagai, A.J. Hobbs, J. Lin, T. Ida, T. Akaike, Biological hydropersulfides and related polysulfides - a new concept and perspective in redox biology, FEBS Lett. 592 (2018) 2140–2152.
[29] H. Kimura, Signalling by hydrogen sulfide and polysulfides via protein S-sulfuration, Br. J. Pharmacol. 177 (2020) 720–733.
[30] H.J. Sun, Z.Y. Wu, X.W. Nie, J.S. Bian, Role of hydrogen sulfide and polysulfides in neurological diseases: focus on protein S-Persulfidation, Curr. Neuropharmacol. (2020).
[31] S. Yuan, X. Shen, C.G. Kevil, Beyond a Gasotransmitter: Hydrogen Sulfide and Polysulfide in Cardiovascular Health and Immune Response, Antioxidants & redox signaling, 2017.
[32] N. Nagahara, S. Koike, T. Nirasawa, H. Kimura, Y. Ogasawara, Alternative pathway of H2S and polysulfides production from sulfurated catalytic-cysteine of reaction intermediates of 3-mercaptopyruvate sulfurtransferase, Biochem. Biophys. Res. Commun. 496 (2018) 648–653.
[33] K.R. Olson, E.R. DeLeon, Y. Gao, K. Hurley, V. Sadauskas, C. Batz, G.F. Stoy, Thiosulfate: a readily accessible source of hydrogen sulfide in oxygen sensing, Am. J. Physiol. Regul. Integr. Comp. Physiol. 305 (2013) R592–R603.
[34] R.M. Howard, G.P. Smith, Treatment of calcinosis cutis with sodium thiosulfate therapy, J. Am. Acad. Dermatol. (2020).
[35] N. Laplace, V. Kepenekian, A. Friggeri, O. Vassal, F. Ranchon, C. Rioufol, W. Gertych, L. Villeneuve, O. Glehen, N. Bakrin, Sodium thiosulfate protects from renal impairement following hyperthermic intraperitoneal chemotherapy (HIPEC) with Cisplatin, Int. J. Hyperther. 37 (2020) 897–902.
[36] Y. Mizuta, K. Tokuda, J. Guo, S. Zhang, S. Narahara, T. Kawano, M. Murata, K. Yamaura, S. Hoka, M. Hashizume, T. Akahoshi, Sodium thiosulfate prevents doxorubicin-induced DNA damage and apoptosis in cardiomyocytes in mice, Life Sci. (2020), 118074.
[37] I.T.N. Nguyen, A. Klooster, M. Minnion, M. Feelisch, M.C. Verhaar, H. van Goor, J. A. Joles, Sodium thiosulfate improves renal function and oxygenation in L-NNA-induced hypertension in rats, Kidney Int. 98 (2020) 366–377.
[38] Q. Chen, S. Qi, L. Hocum-Stone, E. Lesnefsky, R.F. Kelly, E.O. McFalls, Preventing myocardial injury following non-cardiac surgery: a potential role for preoperative antioxidant therapy with ubiquinone, Antioxidants 10 (2021).
[39] K. Bersuker, J.M. Hendricks, Z. Li, L. Magtanong, B. Ford, P.H. Tang, M.A. Roberts, B. Tong, T.J. Maimone, R. Zoncu, M.C. Bassik, D.K. Nomura, S.J. Dixon, J. A. Olzmann, The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis, Nature 575 (2019) 688–692.
[40] C. Guo, F. Liang, M.W. Shah, X. Yan, Hydrogen sulfide protected gastric epithelial cell from ischemia/reperfusion injury by Keap1 s-sulfhydration, MAPK dependent anti-apoptosis and NF-kappaB dependent anti-inflammation pathway, Eur. J. Pharmacol. 725 (2014) 70–78.
[41] J.M. Hourihan, J.G. Kenna, J.D. Hayes, The gasotransmitter hydrogen sulfide induces nrf2-target genes by inactivating the keap1 ubiquitin ligase substrate adaptor through formation of a disulfide bond between cys-226 and cys-613, Antioxidants Redox Signal. 19 (2013) 465–481.
[42] S. Koike, Y. Ogasawara, N. Shibuya, H. Kimura, K. Ishii, Polysulfide exerts a protective effect against cytotoxicity caused by t-buthylhydroperoxide through Nrf2 signaling in neuroblastoma cells, FEBS Lett. 587 (2013) 3548–3555.
[43] W. Meng, Z. Pei, Y. Feng, J. Zhao, Y. Chen, W. Shi, Q. Xu, F. Lin, M. Sun, K. Xiao, Neglected role of hydrogen sulfide in sulfur mustard poisoning: keap1 S-sulfhydration and subsequent Nrf2 pathway activation, Sci. Rep. 7 (2017) 9433.
[44] G. Yang, K. Zhao, Y. Ju, S. Mani, Q. Cao, S. Puukila, N. Khaper, L. Wu, R. Wang, Hydrogen sulfide protects against cellular senescence via S-sulfhydration of Keap1 and activation of Nrf2, Antioxidants Redox Signal. 18 (2013) 1906–1919.
[45] X. Zhou, L. Zhao, J. Mao, J. Huang, J. Chen, Antioxidant effects of hydrogen sulfide on left ventricular remodeling in smoking rats are mediated via PI3K/Akt-dependent activation of Nrf2, Toxicol. Sci. (2014).
[46] D.R. Linden, J. Furne, G.J. Stoltz, M.S. bdel-Rehim, M.D. Levitt, J.H. Szurszewski, Sulfide quinone reductase contributes to hydrogen sulfide metabolism in murine peripheral tissues but not in the central nervous system, Br. J. Pharmacol. 165 (2011) 2178–2190.
[47] A.E. McDonald, D.V. Gospodaryov, Alternative NAD(P)H dehydrogenase and alternative oxidase: proposed physiological roles in animals, Mitochondrion 45 (2019) 7–17.
[48] T.M. Hildebrandt, M.K. Grieshaber, Redox regulation of mitochondrial sulfide oxidation in the lugworm, Arenicola marina, J. Exp. Biol. 211 (2008) 2617–2623.
[49] S. Völkel, M.K. Grieshaber, Mitochondrial sulfide oxidation in Arenicola marina. Evidence for alternative electron pathways, Eur. J. Biochem. 235 (1996) 231–237.
[50] N. Uchida, K. Suzuki, R. Saiki, T. Kainou, K. Tanaka, H. Matsuda, M. Kawamukai, Phenotypes of fission yeast defective in ubiquinone production due to disruption of the gene for p-hydroxybenzoate polyprenyl diphosphate transferase, J. Bacteriol. 182 (2000) 6933–6939.
[51] K.R. Olson, A case for hydrogen sulfide metabolism as an oxygen sensing mechanism, Antioxidants 10 (2021).
[52] K. Ling, A. Xu, Y. Chen, X. Chen, Y. Li, W. Wang, Protective effect of a hydrogen sulfide donor on balloon injury-induced restenosis via the Nrf2/HIF-1alpha signaling pathway, Int. J. Mol. Med. 43 (2019) 1299–1310.
[53] C. Meng, X. Cui, S. Qi, J. Zhang, J. Kang, H. Zhou, Lung inflation with hydrogen sulfide during the warm ischemia phase ameliorates injury in rat donor lungs via metabolic inhibition after cardiac death, Surgery 161 (2017) 1287–1298.
[54] B.F. Peake, C.K. Nicholson, J.P. Lambert, R.L. Hood, H. Amin, S. Amin, J. W. Calvert, Hydrogen sulfide preconditions the db/db diabetic mouse heart against ischemia-reperfusion injury by activating Nrf2 signaling in an Erk-dependent manner, Am. J. Physiol. Heart Circ. Physiol. 304 (2013) H1215–H1224.
[55] B. Yang, Y. Bai, C. Yin, H. Qian, G. Xing, S. Wang, F. Li, J. Bian, M. Aschner, R. Lu, Activation of autophagic flux and the Nrf2/ARE signaling pathway by hydrogen sulfide protects against acrylonitrile-induced neurotoxicity in primary rat astrocytes, Arch. Toxicol. 92 (2018) 2093–2108.
[56] H.J. Sun, Z.Y. Wu, X.W. Nie, X.Y. Wang, J.S. Bian, Implications of hydrogen sulfide in liver pathophysiology: mechanistic insights and therapeutic potential, J. Adv. Res. 27 (2021) 127–135.
[57] Y. Zhang, J. Gao, W. Sun, X. Wen, Y. Xi, Y. Wang, C. Wei, C. Xu, H. Li, H2S restores the cardioprotective effects of ischemic post-conditioning by upregulating HB-EGF/EGFR signaling, Aging (Albany NY) 11 (2019) 1745–1758.
[58] C. Szabo, Hydrogen sulfide, an endogenous stimulator of mitochondrial function in cancer cells, Cells (2021) 10.

[59] Y. Miyazaki, E. Marutani, T. Ikeda, X. Ni, K. Hanaoka, M. Xian, F. Ichinose, A sulfonyl azide-based sulfide scavenger rescues mice from lethal hydrogen sulfide intoxication, Toxicol. Sci. (2021).
[60] E. Marutani, M. Morita, S. Hirai, S. Kai, R.M.H. Grange, Y. Miyazaki, F. Nagashima, L. Traeger, A. Magliocca, T. Ida, T. Matsunaga, D.R. Flicker, B. Corman, N. Mori, Y. Yamazaki, A. Batten, R. Li, T. Tanaka, T. Ikeda, A. Nakagawa, D.N. Atochin, H. Ihara, B.A. Olenchock, X. Shen, M. Nishida, K. Hanaoka, C.G. Kevil, M. Xian, D. B. Bloch, T. Akaike, A.G. Hindle, H. Motohashi, F. Ichinose, Sulfide catabolism ameliorates hypoxic brain injury, Nat. Commun. 12 (2021) 3108.
[61] C. Szabo, The re-emerging pathophysiological role of the cystathionine-beta-synthase - hydrogen sulfide system in Down syndrome, FEBS J. 287 (2020) 3150–3160.