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Enzyme inactivation by metal-catalyzed oxidation of coenzyme Q_1

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Ubiquinol-1 in aerated aqueous solution inactivates several enzymes – alanine aminotransferase, alkaline phosphatase, Na^+/K^+-ATPase, creatine kinase and glutamine synthetase – but not isocitrate dehydrogenase and malate dehydrogenase. Ubiquinone-1 and/or H_2O_2 do not affect the activity of alkaline phosphatase and glutamine synthetase chosen as model enzymes. Dioxygen and transition metal ions, even if in trace amounts, are essential for the enzyme inactivation, which indeed does not occur under argon atmosphere or in the presence of metal chelators. Supplementation with redox-active metal ions (Fe^{3+} or Cu^{2+}), moreover, potentiates alkaline phosphatase inactivation. Since catalase and peroxidase protect while superoxide dismutase does not, hydrogen peroxide rather than superoxide anion seems to be involved in the inactivation mechanism through which oxygen active species (hydroxyl radical or any other equivalent species) are produced via a modified Haber-Weiss cycle, triggered by metal-catalyzed oxidation of ubiquinol-1. The lack of efficiency of radical scavengers and the almost complete protection afforded by enzyme substrates and metal cofactors indicate a 'site-specific' radical attack as responsible for the oxidative damage.

Introduction

Metal-catalyzed oxidation (MCO) reactions are known to inactivate enzymes and modify proteins, thereby serving as marking steps for protein degradation [1–7]. Enzymic and nonenzymic MCO systems share common features. In any case, they require a redox-active transition metal (albeit in trace amounts), oxygen as a source of oxidizing equivalents and a suitable electron donor. In the oxidative process, the electron donor reduces the metal catalyst and produces H_2O_2 [1–4]. The interaction between reduced metal and H_2O_2 on the target enzyme gives place to a 'site-specific' production of yet unidentified active oxygen species (viz. hydroxyl radical ($OH^\cdot$), Fe (IV) Cu(III), singlet oxygen) which oxidize the side-chains of catalytically essential amino-acid residues, thereby inactivating the enzyme [1–5].

Isoprenoid quinones (CoQ_n) have been recognized as redox components of electron transport chains [8,9]. Besides this well-recognized role, several recent reports suggest the implication of coenzyme Q_{10} and its shorter chain derivatives in the protection of membranes from peroxidative damage [10–13] as well as in the production of damaging free radicals [10,11,14].

In view of this, the effect of the ubiquinol-1 (CoQ_1H_2) on several enzyme activities has been investigated. Our results show that the metal-catalyzed oxidation of CoQ_1H_2 causes enzyme inactivation by a 'site-specific' radical attack, thus mimicking the effect of better-known MCO systems.

Materials and Methods

Materials

Materials were purchased from the following sources: EDTA disodium salt from Merck; $FeCl_3 \cdot 6H_2O$ and $CuSO_4$ from Fluka; bovine serum albumin from Serva; $ZnSO_4$, $CoSO_4$ and $MgSO_4$ from Pura-

Abbreviations: MCO, metal-catalyzed oxidation; $OH^\cdot$, hydroxyl radical; $O_2^{\cdot-}$, superoxide anion; CoQ_1H_2, ubiquinol-1; CoQ_1, ubiquinone-1; $CoQ_1^{\cdot-}$, ubisemiquinone-1; AP, alkaline phosphatase; GS, glutamine synthetase; CK, creatine kinase; ALAT, alanine aminotransferase; MDH, malate dehydrogenase; ICDH, isocitrate dehydrogenase; SOD, superoxide dismutase; BSA, bovine serum albumin; DTPA, diethylenetriaminepentaacetic acid; DFO, desferrioxamine; p-NPP, *p*-nitrophenyl phosphate, 4-MUP, 4-methylumbelliferyl phosphate.

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tronic Johnson Matthey Chemicals; spectrographically pure ethanol from Carlo Erba; Coomassie blue G-250 from LKB; Sephadex G-25 from Pharmacia; desferal (deferoxamine methanesulfonate) from Ciba Laboratories; Tris, malate dehydrogenase (from pig heart mitochondria), isocitrate dehydrogenase (from pig heart), alanine aminotransferase (from pig heart) and catalase (from bovine liver) from Boehringer; alkaline phosphatase (from bovine kidney), Na^+/K^+-ATPase (from dog kidney), creatine kinase (from bovine muscle), peroxidase (from horseradish), superoxide dismutase (from bovine erythrocytes) from Sigma. All enzyme proteins were extensively dialyzed prior to use. Coenzyme Q_1 was kindly donated by Eisai. All other chemicals were obtained from Sigma and were of the highest grade available.

Ferric chloride, dissolved in H_2O, was prepared fresh and used immediately. All buffer solutions were prepared in highly purified water (resistivity = 18 MΩ cm) obtained through a Milli-Q water purification system (Millipore). Transition metals were present as a result of trace contaminants in the buffer salts (atomic absorption measurements revealed that iron and copper contamination was no greater than 0.3 μM and no greater than 0.1 μM, respectively). No further attempts were made to lower the level of trace metals in the buffer solution because of the ineffectiveness of the customary precautions for their complete removal [15] and because trace metals were ultimately required in our experiments.

Methods

Protein determination. Protein content was determined using the dye-binding method of Bradford [16] with bovine serum albumin as standard.

Enzyme activity assays. Alkaline phosphatase was purified and assayed as already reported [17]. Activity of glutamine synthetase was assayed by the glutamyl transferase method [5]. Creatine kinase, alanine aminotransferase, malate dehydrogenase and isocitrate dehydrogenase activities were measured according to standard procedures [18]; Na^+/K^+-ATPase according to Ref. 19. Superoxide dismutase (SOD) (3000 units/mg), catalase (65000 units/mg), devoid of superoxide dismutase activity, and peroxidase (170 units/mg) activity were determined as described [17].

Preparation of reduced coenzyme Q_1. Coenzyme Q_1 (CoQ_1) (extinction coefficient of 13.7 mM^{-1} cm^{-1} at 275 nm in absolute ethanol [20]) was reduced using the method described by Rieske [21]. Stock solution of ubiquinol (extinction coefficient of 4.0 mM^{-1} cm^{-1} at 290 nm [20]) was stored in the dark at −30°C, in absolute ethanol and slightly acidic conditions (10 mM HCl). Quinol solutions were stable for several weeks.

Enzyme inactivation experiments. Enzyme preparations (final concentrations are: 0.1 μM alkaline phosphatase (AP), 2 μM (subunit equivalents) glutamine synthetase (GS), 0.25 μM Na^+/K^+-ATPase, 0.56 μM creatine kinase (CK), 0.11 μM alanine aminotransferase (ALAT), 0.35 μM malate dehydrogenase (MDH) and 0.2 μM isocitrate dehydrogenase (ICDH)) were incubated at 37°C in the respective buffer (50 mM Tris-HCl (pH 7.4) for AP, Na^+/K^+-ATPase and ALAT; 50 mM Hepes (pH 7.4) for GS, CK, MDH and ICDH). After equilibration at 37°C (5 min) the reaction was started by the addition of CoQ_1H_2 (0.8 mM final concentration) to enzyme solution. At the desired time, aliquots were withdrawn from the reaction mixture and diluted with the assay mixture, and the residual enzyme activity was determined. The same amount of ethanol-HCl, never exceeding 2% of final volume, was always added to the controls and per se did not modify enzymic activities. Except where noted, the reaction mixtures were not supplemented by redox-active transition metals, since trace metals present in the solution were sufficient for the enzyme inactivation. Half-times for inactivation were determined from a linear least-squares analysis ($r^2 \geq 0.990$) of semilogarithmic plots of residual activity versus time.

Anaerobic experiments were performed in test-tubes fitted with rubber caps into which two needles were inserted to serve as inlet and outlet ports for introduction of argon [5]. After bubbling argon through the incubation mixture for 10 min, the reaction was started by addition of CoQ_1H_2 by means of a gas-tight Hamilton microsyringe.

Protection experiments. In such experiments, enzyme was preincubated with or without a protecting agent (radical scavenger or metal cofactor or substrate). After 10 min incubation, CoQ_1H_2 (0.8 mM final concentration) was added into the reaction mixtures, which were further incubated at 37°C for 60 min. Enzyme activity of each mixture was determined immediately prior to coenzyme addition (time zero) and after 60 min incubation.

Polarographic experiments. Oxygen consumption experiments were performed with a Clark-type electrode on a YSI Model 53 oxygen monitor (Yellow Springs Instrument) equipped with a thermostatted water-jacketed reaction chamber (total volume 3.0 ml) containing a small magnetic stirrer. O_2 consumption was measured in 50 mM Tris-HCl buffer, equilibrated with air at 37°C and assumed to contain the same oxygen concentration as water (207 μM at 37°C). The oxygraph was calibrated daily with a solution of water equilibrated with air for 2 h at 37°C.

Spectrophotometric measurements of CoQ_1H_2 oxidation. The oxidation of 0.8 mM CoQ_1H_2 at 37°C, in 50 mM Tris-HCl buffer, was monitored by the change in absorbance at 410 nm due to CoQ_1 formation. The maximum rate of CoQ_1 formation was calculated according to Ref. 22 using a molar absorption coefficient

of 480 at 410 nm. Absorbance was measured with a Hewlett-Packard 8450 UV/vis spectrophotometer equipped with a cuvette stirring apparatus and a constant-temperature cell holder.

pH studies. pH was measured with a PHM 84 Research pH-meter (Radiometer) fitted with a combination electrode; the electrode response was corrected for temperature.

Other details are given in the results and in the legends of tables and figures.

Results and Discussion

Enzyme inactivation by CoQ_1H_2

Several enzymes which are known to undergo oxidative modification by MCO systems [5,23] are readily inactivated by CoQ_1H_2 at 37°C, pH 7.4, under aerobic conditions. Inactivation always follows pseudo-first-order kinetics and half-lives of tested enzymes are reported in Table I. Conversely, malate dehydrogenase and isocitrate dehydrogenase, already reported as not susceptible to oxidative damage [5,24], are not inactivated by CoQ_1H_2.

Mechanism of enzyme inactivation by CoQ_1H_2

To further characterize CoQ_1H_2-mediated inactivation reactions, alkaline phosphatase and glutamine synthetase are employed as model enzymes, since these proteins are particularly susceptible to metal-catalyzed oxidative modifications [1–5,17,23,25,26]. Enzyme inactivation is not reversible; neither alkaline phosphatase nor glutamine synthetase activity was restored either by passage through a Sephadex G-25 column or after dialysis for 72 h with seven changes of the respective buffer.

Dioxygen is strictly required, since no AP or GS activity decrease can be observed when the reaction mixture is kept under argon atmosphere (Table II).

CoQ_1H_2 in oxygenated aqueous solutions undergoes spontaneous oxidation to CoQ_1 yielding superoxide anion ($O_2^{\cdot-}$) and H_2O_2 [22,27]. Neither CoQ_1 nor hydrogen peroxide, alone or in combination, affects AP and GS activity (Table II); both enzymes, however, are notably insensitive to hydrogen peroxide or to singlet oxygen-generating systems [1–5,23,25,26] Conversely, the addition of H_2O_2 in the reaction mixture before CoQ_1H_2 leads to a significant diminution of AP and GS inactivation, 36 and 23%, respectively (Table II). Superoxide anion radical, at the same time, does not seem to be implicated in the inactivation mechanism, for superoxide dismutase does not protect alkaline phosphatase as well as glutamine synthetase, but rather enhances GS activity decay. Moreover, ubisemiquinone ($CoQ_1^{\cdot-}$), a radical intermediate produced during CoQ_1H_2 oxidation, does not appear the enzyme-damaging species as AP inactivation progressively decreases just in the pH region (see Fig. 4) where the steady-state level of $CoQ_1^{\cdot-}$ increases [28]. On the other hand, catalase and peroxidase protection is consistent with H_2O_2 involvement in CoQ_1H_2-mediated enzyme inactivation, while BSA inefficiency excludes a bulk protein effect (Table II).

Transition metal ions are essential for the inactivation mechanism. In fact, metal chelators such as DTPA, EDTA and desferrioxamine (DFO) block the activity decay almost completely (Table III), although this effect can not be clearly evaluated with the former compounds in the case of AP, the activity of which is strongly affected by the chelation of metal cofactors as magnesium ions. Moreover, addition of Fe(III) or Cu(II) potentiates coenzyme action, increasing the rate of AP inactivation (Fig. 1). As shown in Fig. 1, Cu^{2+} provokes a biphasic effect: an initial, marked acceleration, followed by a progressive decrease in activity decay, which stops after about 10 min. This pattern can

TABLE I

Enzyme inactivation by CoQ_1H_2

Experimental conditions were described under Materials and Methods.

Enzymes inactivated by CoQ_1H_2	Half-life (min)
Alkaline phosphatase	59
Alanine aminotransferase	121
Na^+/K^+-ATPase	74
Creatine kinase	45
Glutamine synthetase	43

TABLE II

Alkaline phosphatase and glutamine synthetase inactivation by CoQ_1H_2

Alkaline phosphatase (in 50 mM Tris buffer (pH 7.4)) and glutamine synthetase (in 50 mM Hepes buffer (pH 7.4)) were incubated at 37°C with the indicated compound(s). After 60 min, enzyme activities were assayed as described under Materials and Methods. Each result is the mean of three separate experiments carried out in duplicate and in each case the values differ by no more than 5%.

	Residual activity after 60 min (%)	
	AP	GS
None	100	100
CoQ_1H_2 (0.8 mM)	50	38
CoQ_1H_2 (0.8 mM) under argon atmosphere	100	100
CoQ_1 (0.8 mM)	100	100
H_2O_2 (1 mM)	100	100
CoQ_1 (0.8 mM) plus H_2O_2 (1 mM)	98	99
H_2O_2 (0.5 mM) plus CoQ_1H_2 (0.8 mM)	68	52
Catalase (4.2 μM) plus CoQ_1H_2 (0.8 mM)	101	99
Peroxidase (4.2 μM) plus CoQ_1H_2 (0.8 mM)	104	102
BSA (4.2 μM) plus CoQ_1H_2 (0.8 mM)	54	45
SOD (4.2 μM) plus CoQ_1H_2 (0.8 mM)	52	29

TABLE III

Effect of metal chelators on enzyme inactivation by CoQ_1H_2

Experimental conditions were as in Table II.

Addition		Residual activity after 60 min (%)	Protection (%)
		Alkaline phosphatase	
None		100	
CoQ_1H_2	(0.8 mM)	50	0
EDTA	(10 μM)	48	
DFO	(1 mM)	100	
DFO	(1 mM) plus CoQ_1H_2 (0.8 mM)	93	86
DTPA	(25 μM)	36	
		Glutamine synthetase	
None		100	
CoQ_1H_2	(0.8 mM)	38	0
EDTA	(1 mM)	100	
EDTA	(1 mM) plus CoQ_1H_2 (0.8 mM)	100	100
DFO	(1 mM)	100	
DFO	(1 mM) plus CoQ_1H_2 (0.8 mM)	98	97
DTPA	(1 mM)	100	
DTPA	(1 mM) plus CoQ_1H_2 (0.8 mM)	99	98

be ascribed to competition for ubiquinol-1 between bound and free copper. Both species, indeed, consume CoQ_1H_2, but, while enzyme-bound copper can trigger a 'site-specific' formation of damaging $OH^{\cdot}$ radicals, unbound Cu^{2+} generates oxyradicals wasting in the bulk. The relatively minor efficiency of ferric iron in catalyzing CoQ_1H_2- as well as ascorbate-mediated [23] enzyme inactivation could be ascribed to the lower solubility and to the lower rate of reduction of Fe^{3+} in respect to Cu^{2+} [29].

Metal chelators, moreover, minimize CoQ_1H_2 oxidation and coenzyme-mediated oxygen consumption; conversely, Fe(III) or Cu(II) supplementation enhances both (Table IV). It should be noted that, in the presence of copper, ubiquinol-1 oxidation is almost complete after about 10 min (84% of CoQ_1H_2 oxidized) accounting for the activity decay profile showed in Fig. 1. The above findings strongly suggest that CoQ_1H_2 reacts with dioxygen at a negligible rate in the absence of a metal catalyst (at least at pH 7.4, see next section) and the process defined as ubiquinol 'autoxidation' is actually a metal-catalyzed oxidation process, analogously to what described for other biomolecules such as ascorbate, glutathione, cysteine, dithiothreitol and so forth [30]. Thermodynamic considerations, based on the redox potentials of the couples ubisemiquinone/ubiquinol ($E^{\circ\prime} = 0.35$ V) ubiquinone/ubisemiquinone ($E^{\circ\prime} = -0.23$ V) [31] and $O_2/O_2^{\cdot-}$ ($E^{\circ\prime} = -0.166$ V) [30], support our data, confirming that, around neutrality, CoQ_1H_2 cannot reduce dioxygen, while the reaction between ubisemiquinone and O_2 is a fast and active exergonic process in aqueous environment [32].

Taken together, our results show that enzyme inactivation by ubiquinol-1 is likely due to oxygen active species (hydroxyl radical or any other highly oxidizing species related to it) produced during the metal-catalyzed oxidation of CoQ_1H_2 rather than to the coenzyme itself or to its oxidation products. According to Scheme 1 (Fig. 2), CoQ_1H_2 oxidation can then generate enzyme-damaging species via a modified Haber-

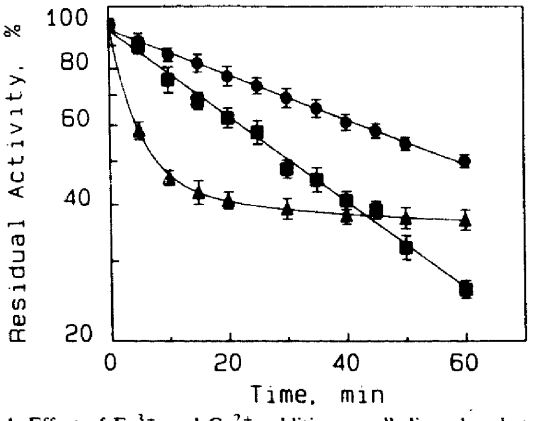

Fig. 1. Effect of Fe^{3+} and Cu^{2+} addition on alkaline phosphatase inactivation by CoQ_1H_2. Alkaline phosphatase (0.1 μM) was incubated with 0.8 mM CoQ_1H_2 alone (●), CoQ_1H_2 plus 100 μM Fe^{3+} (■) and CoQ_1H_2 plus 10 μM Cu^{2+} (▲) under the conditions described under Materials and Methods (CoQ_1H_2 was always the last compound added). Each value is the mean±S.E. of three independent experiments carried out in duplicate.

TABLE IV

Effect of Fe^{3+}, Cu^{2+} and metal chelators on CoQ_1H_2 oxidation

Experimental conditions were described under Materials and Methods and in the legend to Fig. 4.

Addition	Maximum rate of CoQ_1 formation (μM/min)	O_2 consumption (μM/min)
CoQ_1H_2 (0.8 mM)	33.5	22.1
EDTA (25 μM) plus CoQ_1H_2 (0.8 mM)	1.1	3.2
DFO (1 mM) plus CoQ_1H_2 (0.8 mM)	1.0	3.9
DTPA (25 μM) plus CoQ_1H_2 (0.8 mM)	1.9	2.7
Cu^{2+} (10 μM) plus CoQ_1H_2 (0.8 mM)	194.5	87.5
Fe^{3+} (100 μM) plus CoQ_1H_2 (0.8 mM)	72.2	31.9

Scheme 1

$$CoQ_1H_2 \longrightarrow CoQ_1H^- + H^+ \quad (1)$$

$$CoQ_1H^- + M^{n+} \longrightarrow CoQ_1H^{\cdot} + M^{(n-1)+} \quad (2)$$

$$CoQ_1H^{\cdot} \longrightarrow CoQ_1^{\cdot -} + H^+ \quad (3)$$

$$CoQ_1^{\cdot -} + O_2 \longrightarrow CoQ_1 + O_2^{\cdot -} \quad (4)$$

$$2\,O_2^{\cdot -} + 2\,H^+ \longrightarrow H_2O_2 + O_2 \quad (5)$$

$$M^{(n-1)+} + H_2O_2 \longrightarrow Mn^{n+} + OH^{\cdot} + OH^- \quad (6)$$

$$CoQ_1^{\cdot -} + H_2O_2 \longrightarrow CoQ_1 + OH^{\cdot} + OH^- \quad (7)$$

$$CoQ_1H^- + O_2^{\cdot -} + H^+ \longrightarrow CoQ_1 + H_2O_2 \quad (8)$$

$$CoQ_1H^- + H_2O_2 \longrightarrow CoQ_1 + 2\,H_2O \quad (9)$$

$$CoQ_1^{\cdot -} + O_2^{\cdot -} + 2\,H^+ \longrightarrow CoQ_1 + H_2O_2 \quad (10)$$

$$CoQ_1^{\cdot -} + CoQ_1^{\cdot -} + H^+ \longrightarrow CoQ_1 + CoQ_1H^- \quad (11)$$

Fig. 2. Proposed mechanism for hydroxyl radical production by metal-catalyzed oxidation of CoQ_1H_2 at pH 7.4.

Weiss cycle. CoQ_1H_2 indeed reduces transition metal (Fe^{3+} and/or Cu^{2+}), producing ubisemiquinone ($CoQ_1^{\cdot -}$) (reaction 2), which in turn reduces oxygen to superoxide anion that spontaneously dismutes to H_2O_2. The reductive breakdown of H_2O_2 by Fe(II) or Cu(I) ultimately yields $OH^{\cdot}$ radical in a classical Fenton reaction (reaction 6). Nohl and co-workers, however, have suggested that $OH^{\cdot}$ radicals can be also produced by a direct electron transfer from ubisemiquinone ($CoQ_1^{\cdot -}$) to H_2O_2 in a reaction in which the participation of transition metals is not required [14,33]. The latter pathway, although not unlikely, seems more likely

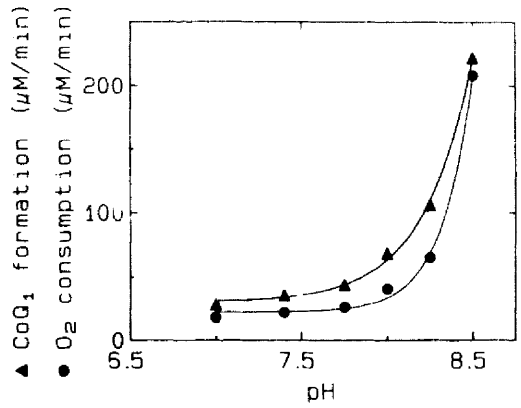

Fig. 4. The pH dependence of CoQ_1H_2 oxidation and CoQ_1H_2-mediated oxygen consumption. The maximum rate of CoQ_1 formation (▲) and oxygen consumption (●) during the oxidation of 0.8 mM CoQ_1H_2 were calculated as described under Materials and Methods. Each value is the mean of three independent experiments carried out in duplicate. S.E. < 10%.

to occur in hydrophobic environments, where ubisemiquinone–dioxygen interaction does not proceed at a significant rate [33–35]. CoQ_1H_2, lastly, can also react with superoxide anion (reaction 7) and/or H_2O_2 (reaction 8). Both reactions consume ubiquinol-1, thus explaining the SOD prooxidant effect as well as the hydrogen peroxide protection observed when either one is present into the reaction mixture before CoQ_1H_2 addition (see Table II).

Effect of pH on alkaline phosphatase inactivation by CoQ_1H_2

AP inactivation by CoQ_1H_2 is dependent on pH, linearly decreasing from pH 7 to pH 9.5 (Fig. 3), while

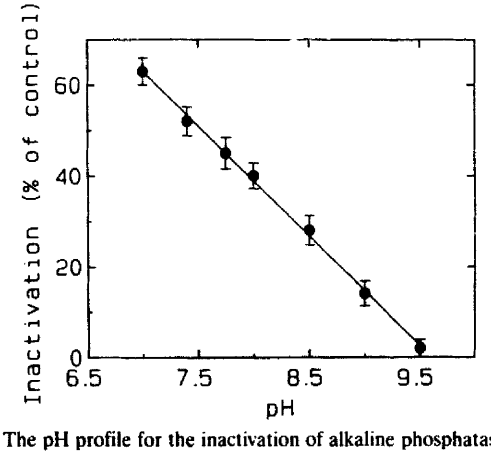

Fig. 3. The pH profile for the inactivation of alkaline phosphatase by CoQ_1H_2. Alkaline phosphatase (0.1 μM) was incubated with 0.8 mM CoQ_1H_2 at 37°C in 50 mM Tris-HCl buffer. Enzyme activity was assayed along with the respective control (enzyme with buffer alone) after 60 min incubation. Each value is the mean ± S.E. of three independent experiments carried out in duplicate.

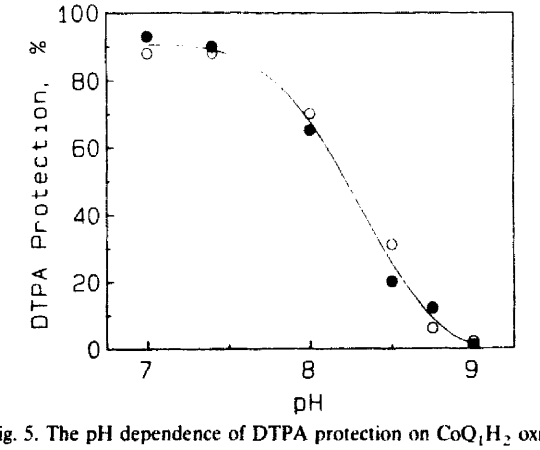

Fig. 5. The pH dependence of DTPA protection on CoQ_1H_2 oxidation and on CoQ_1H_2-mediated oxygen consumption. The protection exerted by DTPA on the maximum rate of CoQ_1 formation (●) and oxygen consumption (○) during the oxidation of 0.8 mM CoQ_1H_2 was expressed as: $[100-(V_c/V_a \times 100)]$ where V_c is the rate in the presence and V_a in the absence of 25 μM DTPA. Experimental conditions were as in Fig. 4.

TABLE V

Effect of radical scavengers and substrates on enzyme inactivation by CoQ_1H_2

Experimental conditions were described under Materials and Methods. Each value is the mean of three independent experiments carried out in duplicate, S.E. < 5%.

Compound added before CoQ_1H_2	Alkaline phosphatase		Glutamine synthetase	
	activity after 60 min (%)	protection (%)	activity after 60 min (%)	protection (%)
None	50	0	38	0
Ethanol (1 M)	50	0	38	0
Formate (0.1 M)	53	6	40	3
Dimethyl sulfoxide (0.1 M)	54	8	36	0
p-NPP (1 mM)	87	74		
4-MUP (1 mM)	90	80		
Phosphate (1 mM)	81	62		
ATP (1 mM)	93	86	40	3
Glutamate (50 mM)			23	−24
ATP (1 mM) plus glutamate (50 mM)			83	73

the rate of CoQ_1H_2 oxidation as well as the coenzyme-mediated oxygen consumption increases with a sigmoidal pattern over the same pH range (Fig. 4). In more alkaline conditions (above pH 7.5) furthermore, the protective effect of DTPA on both O_2 consumption and ubiquinol-1 oxidation quickly diminishes (Fig. 5), suggesting that CoQ_1H_2 oxidation gradually turns into a metal-independent process. Increasing pH above 8 (see again Fig. 4), the amount of CoQ_1H_2 which directly reacts with oxygen also increases (a progressive drop of the redox potential of the ubisemiquinone/ubiquinol couple makes CoQ_1H_2 autoxidation even more thermodynamically favored at higher pH values [28,36]), with a consequent diminution of coenzyme-mediated metal reduction. Since the production of enzyme damaging species is strictly dependent on metal reduction, the above mechanism can account for the progressive reduction of enzyme inactivation with increasing pH.

Effect of radical scavengers, enzyme substrates and metal cofactors

Hydroxyl radical scavengers such as formate, ethanol and dimethyl sulfoxide fail to protect alkaline phosphatase and glutamine synthetase from CoQ_1H_2 inactivation (Table V). On the other hand, substrates (see again Table V) and metal cofactors (Table VI), when incubated with AP before CoQ_1H_2 addition, almost completely block the activity decay. As far as glutamine synthetase is concerned, in the presence of only one substrate enzyme inactivation is either unaffected (see ATP) or slightly stimulated (see glutamate) (Table V), but when both ATP and glutamate are present in the reaction mixture, glutamine synthetase is largely protected from CoQ_1H_2 inactivation (see again Table V). A substrate effect of like nature has been previously described for the oxidative inactivation of glutamine synthetase by ascorbate system [25]. From these results we may infer that 'site-specific' radical damage is responsible for the CoQ_1H_2-mediated enzyme inactivation, similarly to other metal-catalyzed oxidative modifications of proteins.

TABLE VI

Effect of metal cofactors on alkaline phosphatase inactivation by CoQ_1H_2

For details see under Materials and Methods.

Metal cofactor added before CoQ_1H_2	Residual activity after 60 min (%)	Protection (%)
None	50	
Zn^{2+} (2 mM)	95	90
Mg^{2+} (2 mM)	97	94
Co^{2+} (2 mM)	92	84

In conclusion, considering the experimental conditions employed here (CoQ_1 in place of the physiological CoQ_{10}), the potential biological roles of ubiquinol-mediated enzyme inactivation remain speculative. In this regard, some reports have recently highlighted the free-radical-scavenging activity of CoQ [11–13], which is apparently in contrast with our results. It should be recalled, however, that several other biomolecules (i.e., ascorbic acid, α-tocopherol, uric acid) with an unequivocal 'antioxidant reputation' are also able to trigger damaging oxidative reactions, depending on environmental conditions, particularly on metal ion availability [30,37,38].

In our opinion, the close similarity between the oxidative inactivation reactions catalyzed by the CoQ_1H_2 system (CoQ_1H_2/oxygen/trace metals) and

those mediated by better-known MCO systems is therefore tempting and may be a cue for further investigations.

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