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Registry No. Ub, 60267-61-0; ATP, 56-65-5; 2′-deoxy-ATP, 1927-31-7; ITP, 132-06-9; GTP, 86-01-1; 2′-deoxy-GTP, 2564-35-4; etheno-ATP, 37482-17-0; UTP, 63-39-8; CTP, 65-47-4; etheno-CTP, 56405-86-8; $ADPCH_2P$, 3469-78-1; ADPNHP, 25612-73-1; ATPγS, 35094-46-3; ATPβS (S_p), 59261-36-8; ATPβS (R_p), 59261-35-7; ATPαS (S_p), 58976-48-0; ATPαS (R_p), 58976-49-1; PP_i, 14000-31-8; phosphate, 14265-44-2; arsenate, 15584-04-0; methyl phosphate, 512-56-1; tripolyphosphate, 14127-68-5.

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Effects of 2-Hydroxy-3-undecyl-1,4-naphthoquinone on Respiration of Electron Transport Particles and Mitochondria: Topographical Location of the Rieske Iron–Sulfur Protein and the Quinone Binding Site†

H. J. Harmon* and V. G. Struble

ABSTRACT: 2-Hydroxy-3-undecyl-1,4-naphthoquinone is a quinone analogue that inhibits mitochondrial respiration in the cytochrome b–c_1 region with an apparent K_i of 2.5×10^{-7} M. In electron transport particles, it prevents the reduction of cytochrome c_1 by succinate but not its oxidation by oxygen and prevents oxidation of cytochrome b but not its reduction by succinate. The analogue increases the amount of steady-state cytochrome b reduction in actively respiring particles. It inhibits oxidant-induced reduction of cytochrome b in the presence of antimycin. Inhibition of succinate oxidase activity in electron transport particles is independent of the pH of the suspending medium while at pH values above 8 with mitochondria, inhibition decreases. Since the apparent pK of the bound quinone is pH 6.6, the pH dependency of the inhibition is likely due to the pK of the Rieske iron–sulfur center (pH 8). The Rieske center and thus the quinone binding site are located on the cytoplasmic face of the inner membrane.

The second site of oxidative phosphorylation is associated with the ubiquinol–cytochrome c reductase portion of the mitochondrial electron transport chain. This complex, known as complex III (Hatefi et al., 1962), is also responsible for energy-linked proton translocation from the matrix to the cytoplasmic surface (Alexandre et al., 1980). Four electron carriers [two b-type cytochromes, cytochrome c_1, and the iron–sulfur center described by Rieske (Rieske et al., 1964a,b)] and ubiquinone are arranged in the membrane to allow vectorial proton translocation and generation of membrane potential. This arrangement is known as the protonmotive Q cycle (Mitchell, 1975; Bowyer & Trumpower, 1981).

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Description of electron transport and proton translocation via the Q cycle necessitates definition of the location of the redox carriers in the mitochondrial inner membrane. Numerous studies utilizing impermeant probes (Bell et al., 1979; Gellerfors & Nelson, 1977; Nelson & Mendel-Hartvig, 1977) or diffraction techniques (Leonard et al., 1981) agree that complex III physically spans the membrane.

Considerable interest surrounds the function and location of the Rieske iron-sulfur protein. Quinone analogues have been used to demonstrate the dependence of electron flow in complex III on the Fe-S center and to verify the function of the Q cycle (Trumpower & Haggerty, 1980; Bowyer et al., 1982; Trumpower, 1981). Implicit in this description is the existence of the center on the cytoplasmic face. Labeling of yeast mitochondria and submitochondrial particles with diazobenzene[^{35}S]sulfonate locates the center on the C side (Beattie et al., 1981). Similar data have been suggested by Bell et al. (1979) in beef heart mitochondria. Grigolova & Konstantinov (1977) have determined the existence of a 2,3-dimercaptopropanol- (BAL) sensitive center on the C side of the membrane while Slater & de Vries (1980) and Slater et al. (1981) have indicated that BAL reacts with the Rieske center.

Ohnishi et al. (1982) have investigated the effects of dysprosium complexes on the electron paramagnetic resonance (EPR) characteristics of the isolated Rieske center and of the center in mitochondria and electron transport particles. They report that the iron cluster is 19 Å from the surface of the protein and 19-20 Å from the C side of the membrane; the EPR signal is unaffected in electron transport particles, indicating that the center is located on or near the cytoplasmic face. This is in agreement with the studies of Case & Leigh (1976) on the effects of Gd^{2+} and Ni^{2+} on paramagnetic centers of complex III in pigeon heart mitochondria and submitochondrial particles.

In this study, we utilize the exposure to the medium of opposite membrane faces of intact beef heart mitochondria and electron transport particles. On the basis of differences in pH-dependent inhibition of succinate oxidase by 2-hydroxy-3-undecyl-1,4-naphthoquinone (HUNQ), the Rieske center is determined to be on the cytoplasmic face of the inner membrane.

Materials and Methods

Electron transport particles (ETP) were isolated from beef heart mitochondria by the procedure of Crane et al. (1956). Intact beef heart mitochondria were isolated by the procedure of Harmon & Crane (1973). The extent of inversion of the ETP was determined by the lack of stimulation of succinate oxidase by addition of exogenous cytochrome *c* as described previously (Harmon & Crane, 1976). Succinate oxidase activity was measured polarographically at 25 °C as previously described (Harmon & Crane, 1976), except that 27.8 mM 4-morpholineethanesulfonic acid-tris(hydroxymethyl)aminomethane (Mes-Tris) buffer at the desired pH value was used in place of phosphate-ethylenediaminetetraacetic acid (EDTA) buffer in a total volume of 1.8 mL.

2-Hydroxy-3-undecyl-1,4-naphthoquinone (HUNQ) was purchased from Aldrich Chemical Co. Absolute spectra of HUNQ in 50% ethanol solution were recorded with a Cary 14 spectrophotometer against a 50% ethanol blank.

Difference spectra of electron transport particles and double-beam kinetics of cytochromes *b* (564 minus 575 nm wavelength pair) and cytochromes *c* plus c_1 (554 minus 540 nm) were recorded on a Johnson Foundation (University of Pennsylvania, Philadelphia, PA) DBS-3 scanning double-beam spectrophotometer with a magnetically stirred cuvette.

Cytochrome oxidase and succinate-indophenol reductase activities were measured as described by Harmon & Crane (1976). Ferrocyanide oxidase activity was measured as described by Harmon & Basile (1982).

Results

pK of HUNQ. HUNQ is an analogue of UHDBT (5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole) and may be expected to inhibit respiration in a similar manner, namely, by binding at the site of the Rieske iron-sulfur center in place of ubisemiquinone (Trumpower, 1981). It may also be expected to have physical characteristics similar to those of UHDBT. Figure 1A illustrates the neutral and anionic forms of oxidized HUNQ in 50% ethanol solution at pH 4.4 and 8.5, respectively. Prominent absorbance peaks are observed at 252, 283, and 334 nm at acid pH and 231, 276, and 495 nm at alkaline pH values. As shown in Figure 1B, the amplitude of the 495-nm peak, like that of the other peaks (not shown), varies with the pH of the solution, exhibiting an apparent p*K* of approximately pH 6.6. The pink color characteristic of the presence of anionic naphthoquinone (Ball, 1936) is not observed visually below pH 6.3 and is quite prominent above pH 6.5, further corroborating the p*K* value of 6.6. Data in Figure 1C indicate that the p*K* of HUNQ in the presence of oxidized (not shown) or reduced mitochondria is also 6.6. This is very similar to the p*K* value of UHDBT (pH 6.5; Trumpower & Haggerty, 1980) and bound ubisemiquinone in isolated succinate-cytochrome *c* reductase (pH 6.4; Ohnishi & Trumpower, 1980).

Determination of Site of Inhibition. At 6.3×10^{-7} M, HUNQ inhibits less than 5% of succinate-indophenol reductase (thenoyltrifluoroacetone-sensitive succinate dehydrogenase) activity, less than 10% of ascorbate plus TMPD- (*N,N,N'*,-*N'*-tetramethylphenylenediamine) driven cytochrome oxidase activity, and 100% of succinate-cytochrome *c* reductase activity. This indicates that HUNQ inhibits in the cytochrome *b*-*c* region only. In electron transport particles, HUNQ does not inhibit ferrocyanide oxidase mediated via cytochrome c_1 (Harmon & Basile, 1982). Thus, HUNQ inhibits in the b-c_1 region on the substrate side of cytochrome c_1.

To determine the site of inhibition in the b-c_1 region, reduction of cytochromes was measured in ETP with succinate as substrate. As shown in Figure 2A, addition of HUNQ inhibits the reduction of cytochromes *c* plus c_1 by succinate. Addition of HUNQ to succinate-driven anaerobic ETP results in the oxidation of cytochromes *c* plus c_1 (rereduction of cytochromes *c* plus c_1 is not observed), while addition of oxygen in the absence of HUNQ (trace 1) causes a transient oxidation of cytochromes *c* plus c_1. As seen in Figure 2B, addition of HUNQ to succinate-reduced ETP results in oxidation of cytochromes aa_3 and *c* plus c_1 but not cytochromes *b*. When HUNQ is added before succinate (Figure 2C), reduction of cytochrome *b* but not aa_3 or cytochromes *c* plus c_1 is observed. This indicates that HUNQ inhibits respiration on the substrate side of cytochrome c_1 yet prevents oxidation of cytochrome *b*; in this aspect, it is identical in action with UHDBT.

That HUNQ inhibits oxidant-induced reduction of cytochrome *b* is shown in Figure 3. Panel A illustrates the reduction of approximately 50% of cytochrome *b* by succinate upon anaerobiosis; addition of oxygen and antimycin results in the reduction of virtually all of the remaining cytochrome *b*. If antimycin (panel B) is added before succinate, immediate (oxidant-induced) reduction of cytochrome *b* is observed. If HUNQ is added prior to addition to antimycin (plus O_2) and succinate (panel C), only 70% reduction of cytochromes *b* is

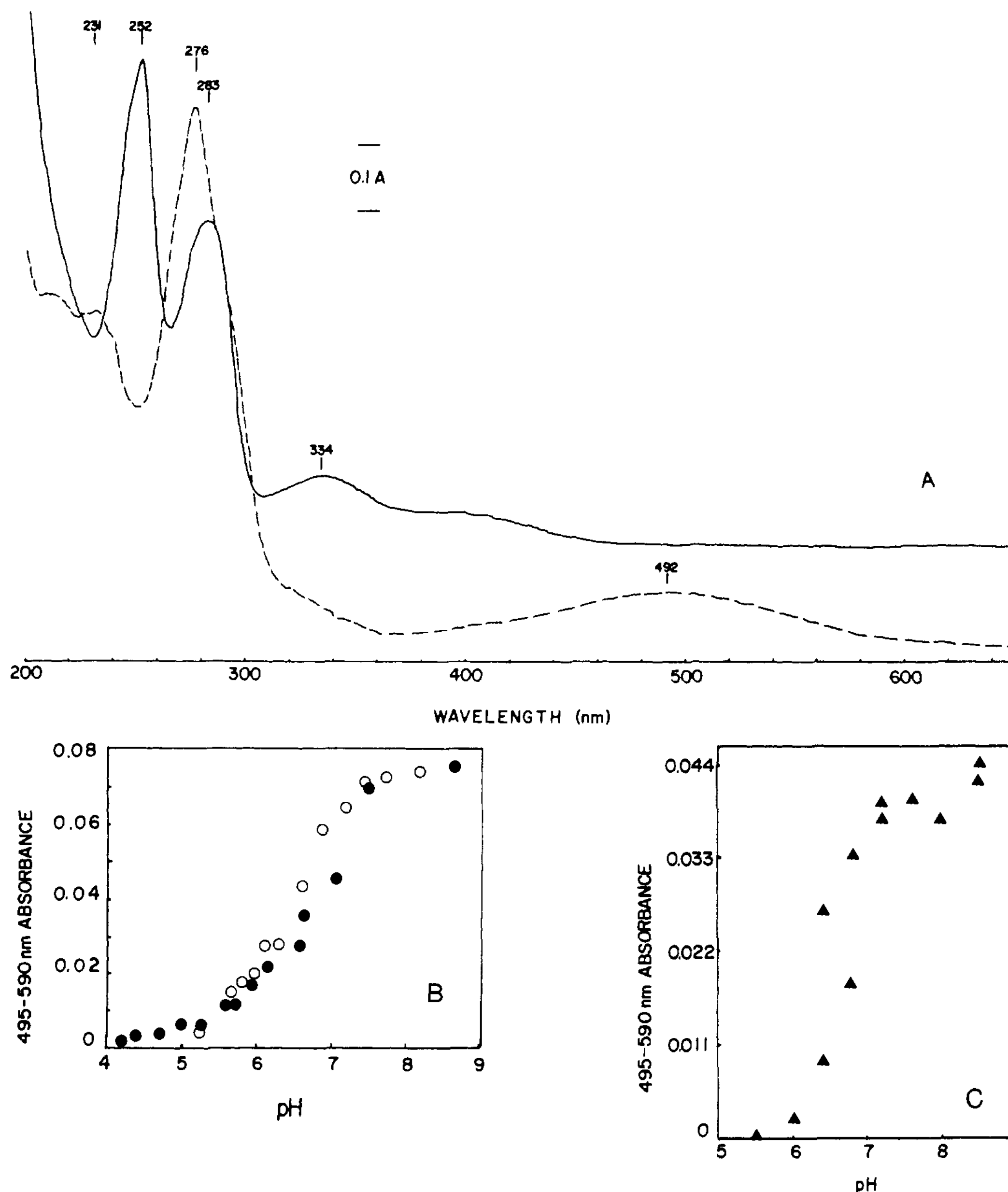

FIGURE 1: (A) Absorbance spectrum of 2-hydroxy-3-undecyl-1,4-naphthoquinone (0.01 mg/mL) in 50% ethanol at pH 8.5 (dashed line) and 4.4 (solid line). pH was adjusted with NaOH or acetic acid, respectively. (B) 495–590-nm absorbance as a function of pH. The cuvette contained 0.01 mg of HUNQ/mL in 50% ethanol. Open and solid circles represent two different titrations. An apparent pK is observed at approximately pH 6.6. Deviation from the expected sigmoid curve in the range of pH 5.5–6.5 is due to the tendency of HUNQ to precipitate from solution at those pH values. (C) 495–590-nm absorbance of HUNQ in the presence of reduced mitochondria as a function of pH. Mitochondria (1.5 mg/mL) were suspended in 0.025 M Mes–Tris buffer at the desired pH and reduced with dithionite. Absorbance (495–590 nm) was measured in the presence of 4.53×10^{-6} M HUNQ against an ethanol control. The HUNQ/protein ratio was the same as that in Figures 4 an 5 at HUNQ concentrations corresponding to 1.7×10^{-7} M HUNQ.

observed; addition of oxygen does not cause further reduction of cytochrome b although cytochrome b is reducible by dithionite. These results are consistent with HUNQ inhibiting oxidation of the b cytochromes and are in agreement with the findings of Bowyer et al. (1981, 1982) with UHDBT.

Oxidant-induced reduction of cytochrome b requires the presence of a functional Rieske center (Trumpower, 1981; Bowyer et al., 1981; Edwards et al., 1982). Since HUNQ inhibits c_1 reduction but not its oxidation and does not inhibit reduction of cytochrome b by succinate but inhibits oxidant-induced reduction, the site of action is at the Rieske iron–sulfur center. This is in agreement with the recent report of alteration of the Rieske EPR signal by naphthoquinone in *Rhodopseudomonas sphaeroides* (Matsuura et al., 1983).

Effect of pH on Inhibition by HUNQ in ETP and Mitochondria. The effect of HUNQ on succinate oxidase activity was measured in ETP and mitochondria at the approximate pH range of 6–8. At pH values below 7.5, similar concentrations of HUNQ inhibit respiration in ETP and mitochondria to approximately the same extent. Inhibition of 50% occurs at approximately 2×10^{-7} M HUNQ. At pH 8, however, ETP exhibit far more inhibition than do mitochondria; 50% inhibition of succinate oxidase is observed at 2.5×10^{-7} M HUNQ in ETP while more than 9×10^{-7} M HUNQ is needed to inhibit mitochondrial activity. The concentrations of cytochrome b (and Rieske center) in the ETP and mitochondria were identical (0.76 nmol of cytochrome b/mg of protein). Thus at any given concentration of HUNQ, the HUNQ/b ratio is the same in ETP and mitochondria. Concentration differences cannot account for the differences observed in

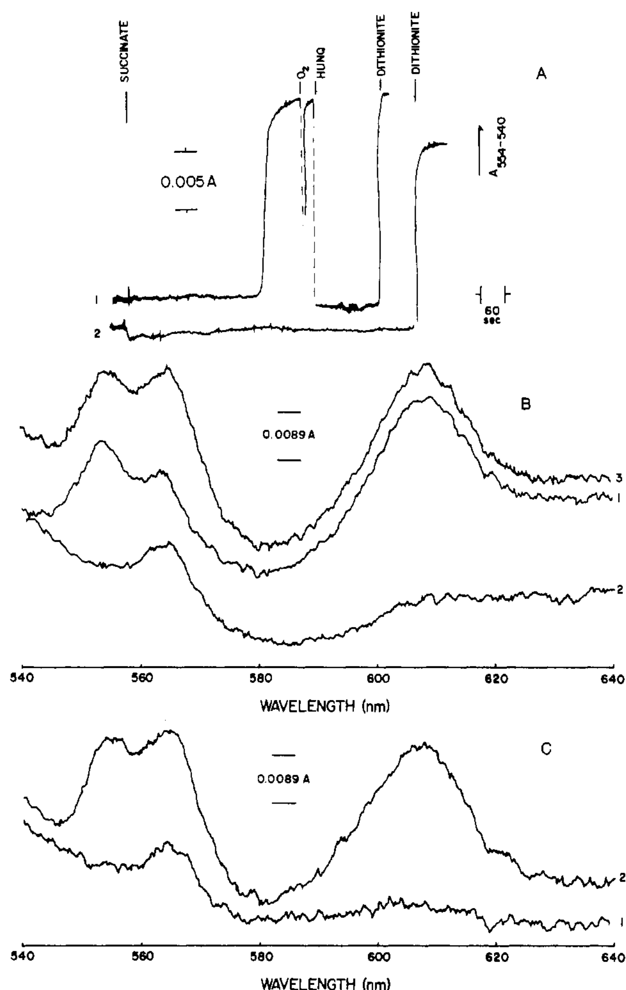

FIGURE 2: (A) Effect of HUNQ on cytochrome c and c_1 oxidation (trace 1) and reduction (trace 2) in electron transport particles. The reaction mixture contained 1.69 mg/mL ETP in 0.25 M sucrose–40 mM Tris–SO_4 (pH 7.4) buffer. Succinate was added to 11.1 mM final concentration. Maximum absorbance of cytochromes c plus c_1 was obtained by addition of solid dithionite. 1.74×10^{-4} M HUNQ was added after succinate addition in trace 1 but immediately prior to addition of succinate in trace 2. (B) Oxidation of cytochromes c plus c_1 and aa_3 on addition of HUNQ. Initial reaction mixture as in Figure 2A. (Curve 1) 11.1 mM succinate added (mixture becomes anaerobic); (curve 2) 1.74×10^{-4} M HUNQ added to curve 1 mixture as in (A); (curve 3) solid excess dithionite added to curve 2 mixture. (C) Effect of HUNQ on cytochrome reduction in ETP. Initial reaction mixture as described in (A). (Curve 1) 1.74×10^{-4} M HUNQ added before addition of 11.1 mM succinate; (curve 2) solid dithionite added.

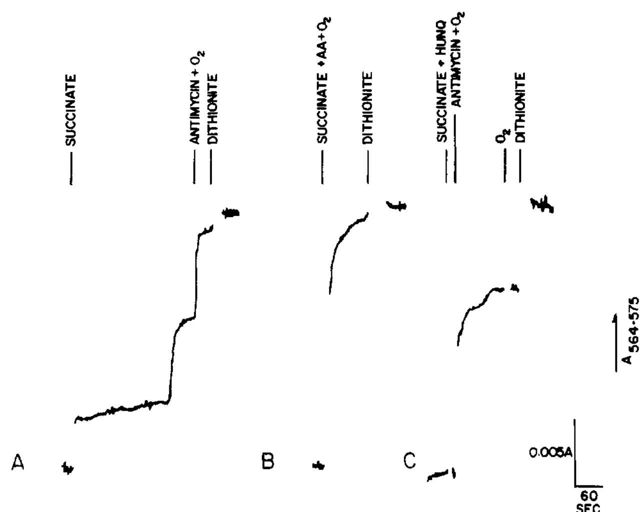

FIGURE 3: Inhibition of oxidant-induced reduction of cytochrome b by HUNQ. 1.82 mg/mL submitochondrial particles [isolated according to Harmon (1982)] were suspended in 0.25 M sucrose–40 mM Tris–SO_4 (pH 7.4) buffer. Succinate (11.1 mM) and antimycin A (100 μg) (1.2×10^{-4} M final concentration) were added where indicated. (Panel A) No addition of HUNQ. Antimycin plus oxygen added after succinate. (Panel B) No HUNQ added, but antimycin (plus O_2) added immediately prior to succinate addition. (Panel C) 1.74×10^{-4} M HUNQ added before antimycin (plus O_2) and succinate.

Table I: Specific Activity of Succinate Oxidase at Various pH Values in Electron Transport Particles and Intact Beef Heart Mitochondria

mitochondria		electron transport particles	
pH	activity[a]	pH	activity[a]
6.0	73	6.0	78
6.68	94.5	6.87	154
7.4	123	7.34	217
7.88	98	7.92	247
8.2	89	8.32	209

[a] nmol of O_2 min^{-1} (mg of protein)$^{-1}$.

Figure 4.

The difference between ETP and mitochondria is more striking if the concentration calculated to give 50% inhibition is plotted vs. pH as in Figure 5. The 50% inhibitory concentration is fairly constant in ETP at all pH values in contrast to mitochondria where a marked increase in HUNQ concentration is needed at high pH values to achieve 50% inhibition of succinate oxidase activity.

Bowyer et al. (1982) have reported that the inhibition of succinate-cytochrome c reductase activity at pH 7.0 is dependent on the respiratory rate; UHDBT is more inhibitory at higher respiratory rates. It is conceivable that the differences in inhibition by HUNQ noted in Figures 4 and 5 are due to differences in respiratory rates of ETP and mitochondria at different pH values. To check this point, the control respiratory rates of ETP and mitochondria at different pH values were determined and are provided in Table I. As can be seen, at virtually every pH value tested, the respiratory rates of ETP exceeded those of mitochondria almost 2-fold, yet the HUNQ concentration needed to yield 50% inhibition (Figure 5) is virtually identical in both particles at pH values below pH 7.4. At more alkaline values one might anticipate the data in Figure 5 if the respiratory rate of mitochondria decreased with increasing pH more than did the respiratory rate of ETP. This is not the case, however. As shown in Figure 6, ETP and mitochondria show similar changes in respiratory rate with pH (using the rates at pH 7–7.4 as 100% standard value). This indicates that the marked decrease in sensitivity of mitochondria to HUNQ at alkaline pH values is not caused by changes in respiratory rates alone since ETP experience a similar decrease in activity without decreased sensitivity to HUNQ.

Discussion

Previous reports (Harmon & Crane, 1973, 1976; Harmon & Basile, 1982) have indicated that the intact mitochondria isolated for this study are physiologically oriented and 95% depleted of endogenous cytochrome c, exhibiting a 16–20-fold stimulation of succinate oxidase activity upon addition of exogenous cytochrome c. On the other hand, the ETP are greater than 94% inverted; exogenous cytochrome c stimulates NADH or succinate oxidase activities less than 6% (Crane et al., 1956; Harmon & Crane, 1976; Harmon & Basile,

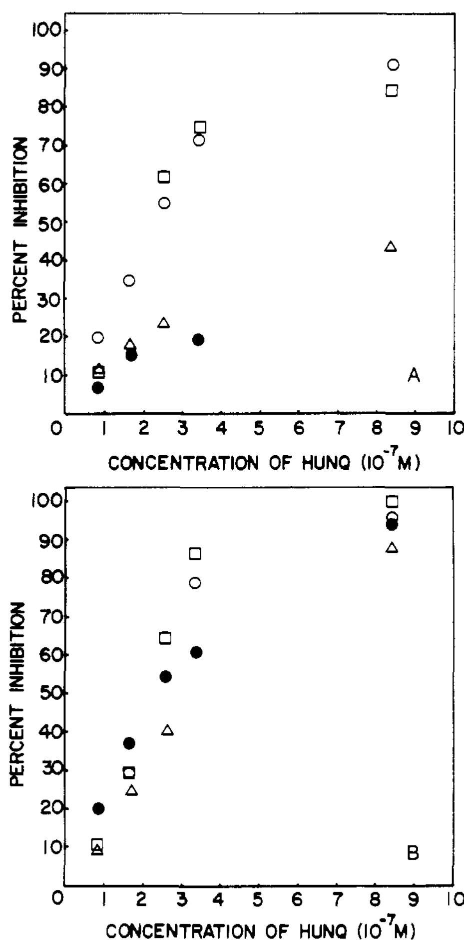

FIGURE 4: Inhibition of succinate oxidase activity by HUNQ in mitochondria and electron transport particles as a function of pH. (A) Inhibition of succinate oxidase activity in intact beef heart mitochondria. 100 μg of mitochondria plus 200 μg (0.92 μM) of cytochrome *c* (Sigma type III) were suspended in 1.8 mL of 27.8 mM Mes–Tris buffer at the indicated pH values. The reaction was initiated by addition of 27.8 mM succinate. The reaction was terminated by addition of 1 μg of antimycin; inhibition of antimycin-sensitive activity is plotted. (Squares) pH 6.7; (open circles) pH 7.4; (triangles) pH 7.9; (closed circles) pH 8.2. (B) Inhibition of antimycin-sensitive succinate oxidase activity in ETP. 100 μg of ETP was suspended in 1.8 mL of 27.8 mM Mes–Tris buffer at the indicated pH values. The reaction was initiated by addition of 27.8 mM succinate. All activity was inhibited by addition of 1 μg of antimycin; inhibition of antimycin-sensitive succinate oxidase activity is plotted. (Open circles) pH 6.0; (squares) pH 7.0; (triangles) pH 7.9; (closed circles) pH 8.3.

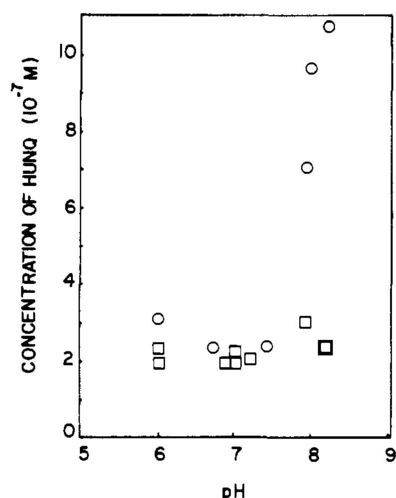

FIGURE 5: Concentration of HUNQ needed to inhibit 50% of succinate oxidase activity in ETP (squares) and mitochondria (circles) at different pH values. Experimental conditions are as described in Figure 4. Data taken from % inhibition vs. HUNQ concentration plots similar to those of Figure 4.

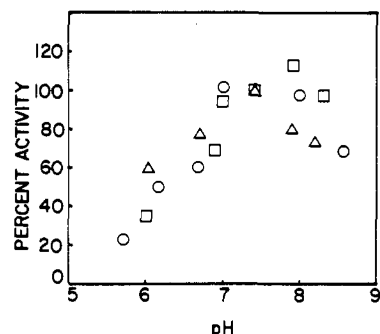

FIGURE 6: Comparison of succinate oxidase activities in mitochondria and ETP at different pH values. The rate at pH 7.4 [123 nmol of O_2 min^{-1} (mg of protein)$^{-1}$] was chosen as the 100% value for mitochondria (triangles). The rate of ETP (squares) at pH 7.34 (100% point) is 217 nmol of O_2 min^{-1} (mg of protein)$^{-1}$. Points denoted by triangles and squares were taken from the data in Table I. Circles denote data from assays performed on the same day with intact mitochondria [159 nmol of O_2 min^{-1} (mg of protein)$^{-1}$ at pH 7.0 used as 100% point]. Data denoted by triangles and squares represents the control rates from experiments in Figures 4 and 5.

1982). Thus the ETP and mitochondria are homogeneously oriented and have the matrix and cytoplasmic surfaces exposed to the medium, respectively.

Ball et al. (1947) reported that some alkylnaphthoquinone derivatives may inhibit succinate dehydrogenase activity. Tappel (1960) indicated that alkylhydroxynaphthoquinones inhibit succinate-ubiquinone reductase at 3 mM naphthoquinone concentration, 4 orders of magnitude greater than that used in these current experiments. It was important to the conclusions drawn in this study to determine that the inhibition of succinate oxidase activity and the observed pH dependency in mitochondria are due to inhibition in the cytochrome b–c_1 region *only*. HUNQ does not inhibit succinate dehydrogenase activity at the HUNQ concentrations at which total inhibition of succinate oxidase occurs. Since cytochrome oxidase activity is also unaffected, the inhibition reported here occurs only in the b–c_1 region as suggested by Roberts et al. (1978).

The difference between the p*K* of the naphthoquinone analogues (cf. Figure 1) and the p*K* of the inhibition of respiration (Figures 4 and 5) has been noted previously by Ball et al. (1947) but unexplained. The curve for inhibition in mitochondria (Figure 5) is similar to that illustrating the pH dependency of respiration of isolated b–c_1 complex with UHDBT. Trumpower & Haggerty (1980) reported the pH dependency to the p*K* of UHDBT and concluded that the ionized form of UHDBT (at alkaline pH values) is not as inhibitory as the protonated form. The results presented here suggest a different interpretation, however.

Data in Figures 4 and 5 indicate that there is not likely to be a difference in the inhibitory potency of the semiquinone QH· compared to the semiquinone anion Q^-· or on the basis of an impermeant form of semiquinone. If either the protonated or anionic forms were more inhibitory, the pH-independent inhibition of respiration in ETP would not be expected. At alkaline pH values the anionic form would increase in concentration; if the anionic form were less potent, then the HUNQ concentration needed for inhibition would increase. According to the p*K* values for ubisemiquinone and ubihydroquinone (pH 6.4 and 8, respectively) published by Ohnishi & Trumpower (1980), the Q^-·/QH_2 ratio at pH 8 is 100-fold greater than at pH 7. If QH· is the inhibitory species or more inhibitory than other species [as has been suggested

(Trumpower & Haggerty, 1980; Bowyer et al., 1982; Trumpower, 1981)], a 100-fold difference in HUNQ concentration needed for inhibition would be observed at these pH values; at lower pH values, 100-fold less HUNQ would be required for each pH-unit decrease. This is not observed. It is therefore likely that the protonated and anionic forms are equally potent.

That an impermeant species of HUNQ is involved in the inhibitions reported in Figures 4 and 5 is also unlikely. If the Rieske center were located on only one membrane face, we would expect the curves in Figures 4 and 5 to reflect significant differences at all pH values greater than the p*K* value. This is not observed; the concentration needed to inhibit respiration is very similar in ETP and mitochondria up to approximately pH 7.5, at which point the ratio of anionic to protonated form is at least 10/1 if the p*K* = 6.5 and 700/1 if the p*K* = 5 as suggested by Ball (1936). If the anionic form were more impermeant than the protonated form, ETP and mitochondria would not show similar concentration dependence below pH 7.5.

That an impermeant species of HUNQ is not the basis of the data presented here is also supported by the observation that 45% inhibition of succinate oxidase activity is observed with submitochondrial particles (Harmon, 1982) suspended at pH 7.9 in the presence of 3.2×10^{-7} M HUNQ, in fair agreement with the data for ETP in Figures 4B and 5. In the presence of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 12% inhibition is observed in the presence of 3.2×10^{-7} M HUNQ, in fair agreement with the data in Figures 4A and 5 for mitochondria. In the absence of uncoupler, the pH of the matrix face is 7.9 while that of the sequestered cytoplasmic face is 7.4 (that of the buffer during sonication); the Rieske center, if on the cytoplasmic face, would be readily inhibited by HUNQ. In the presence of protonophoric uncoupler, the pH of both membrane faces is 7.9; the extent of inhibition decreases because the pH at the Rieske site increases. These data indicate that HUNQ is not impermeant at high pH values but that the reactive site (Rieske) is pH sensitive and located on the cytoplasmic face.

As seen in Figure 6 and Table I, the difference in inhibition in ETP and mitochondria by HUNQ is not due to differences in respiratory rate. At pH 7, the 50% inhibitory concentration of HUNQ is the same for both ETP and mitochondria, yet the respiratory rate of mitochondria is only 60% that of ETP. If the pH dependency in mitochondria were caused by the decreased respiratory rates at alkaline pH values, we would expect that mitochondria at pH 6 and 6.7 and ETP at pH 6 would also exhibit decreased sensitivity to HUNQ at these pH values since the specific activities are comparable. This is not observed, indicating that respiratory rate dependency of inhibition is not a significant contributor to the effect illustrated in Figure 5. If it were a significant factor and if the pH dependency were due to the p*K* of HUNQ, then decreased sensitivity to HUNQ at pH values less than 7 would have been observed. If the differences between ETP and mitochondria (Figure 5) were due to pH-dependent respiratory rate differences, then the 50% inhibitory concentrations at pH values greater than 7 should decrease. They do not.

Of further importance is the observation that while in the presence of uncoupler the extent of inhibition by HUNQ decreases, the rate of respiration [86 nmol of O_2 min^{-1} (mg of protein)$^{-1}$] does not change. Thus, the decrease in sensitivity to HUNQ in Figures 4 and 5 is not due to differences in respiratory rate.

The differences in inhibition in ETP and mitochondria are due to the location of the Rieske center on one membrane face and the exposure of that face to the suspending medium and *not* to the presence of a pH-dependent form of quinone. If the Rieske center is not exposed to either face, then differences would not be observed. The p*K* of the Rieske center in pigeon heart mitochondria is 8 (Prince & Dutton, 1976). At alkaline pH values, the midpotential of the iron–sulfur protein decreases; the Rieske center would be more readily oxidized by cytochrome c_1. Since the quinone binds more readily to the reduced protein (Trumpower, 1981), HUNQ binding would be less, and the extent of inhibition would decrease at high pH values. The particle type that exhibits a decrease in inhibition at high pH has the Rieske center exposed to the medium; thus, the Rieske center is exposed on the C side of the membrane in mitochondria. If the center were on the M side, the decrease in inhibition (Figure 4) would be seen with ETP and not with mitochondria. Decrease in inhibition in ETP is not seen because the Rieske center is on the membrane face within the vesicle. In both ETP and mitochondria, the pH of the intravesicular space is constant even though the pH of the suspending medium changes (Harmon & Basile, 1982). Thus in ETP, the pH of the C side of the membrane, and thus the oxidation state of the Rieske center, is constant. The inhibition in ETP will be pH independent, as observed. In mitochondria, the pH and the oxidation state of the Rieske center change with the pH of the medium; pH-dependent inhibition will be observed.

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Registry No. HUNQ, 41245-59-4; cytochrome *b*, 9035-37-4; cytochrome c_1, 9035-42-1; succinate oxidase, 9014-35-1.

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Transfer Ribonucleic Acid Deprived of the C-C-A 3′-Extremity Can Interact with Elongation Factor Tu†

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ABSTRACT: In this work we describe that uncharged tRNAs or modified tRNAs lacking all or part of the C-C-A end (i.e., tRNA minus pCpCpA, tRNA minus pA, and tRNA minus A) can still influence the GTPase activity of the elongation factor Tu (EF-Tu), thus showing that, besides the aminoacylated 3′-end, other regions of the aa-tRNA interact with EF-Tu. The existence of an interaction between EF-Tu and truncated tRNAs was also confirmed by examining the dissociation of the EF-Tu·GTP complex: the rate of this reaction is decreased upon addition of $tRNA_1^{Val}$ minus pCpCpA. The effect on the EF-Tu GTPase activity of tRNAs deprived of the C-C-A 3′-end is still evident in the presence of C-C-A-aa. The stimulatory pattern obtained with C-C-A-Val at 5 mM $MgCl_2$ is decreased upon addition of $tRNA_1^{Val}$ minus pCpCpA, $tRNA_1^{Val}$ minus pA, or $tRNA_1^{Val}$ minus A. This shows that the effect of the aminoacylated C-C-A 3′-end can be influenced via EF-Tu by the remaining regions of the tRNA, after cleavage of a bond in the 3′-extremity. However, also with an excess of $tRNA_1^{Val}$ minus pCpCpA over C-C-A-Val, no "aa-tRNA-like" effect, i.e., no inhibition of the EF-Tu GTPase, was obtained, suggesting that, upon binding with EF-Tu, a specific conformational change in the aa-tRNA molecule also takes place, regulating the expression of the GTPase activity. Our results unequivocally show that different regions of the aa-tRNA are needed for a coordinated interaction with EF-Tu.

The C-C-A[1] 3′-terminal region plays a fundamental role in tRNA functions, since it is directly involved in interaction with other cellular components. In fact, the 3′-terminal oligonucleotides of tRNA can still interact with other biological macromolecules, such as synthetases (Renaud et al., 1981), ribosomes (Ringer et al., 1976; Kukhanova et al., 1980), or EF-Tu in the ternary complex (Jõnak et al., 1980). Evidence for the existence of a ternary complex between the C-C-A-aa fragments and EF-Tu also results from the observation that such aminoacylated oligonucleotides can stimulate the GTPase activity of EF-Tu (Campuzano & Modolell, 1980; Bhuta & Chlâdek, 1980; Parlato et al., 1981) and inhibit the dissociation of the EF-Tu·GTP complex (O. Fasano, unpublished results).

Recently, we have reported that ionic conditions, particularly a narrow range of Mg^{2+} concentrations, selectively regulate the aa-tRNA effect but not that of short 3′-aminoacylated fragments (Parlato et al., 1983). This suggests that, besides the 3′-extremity, other domains of the aa-tRNA molecule interact with EF-Tu and are involved in the regulation of the GTPase reaction. A suitable approach to clarify this point may consist in comparing the effects of tRNA molecules that completely or partially lack the C-C-A 3′-terminal sequence with that of aa-tRNA or C-C-A-aa alone.

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[1] Abbreviations: EF-Tu, elongation factor Tu; EF-Ts, elongation factor Ts; GTP, guanosine 5′-triphosphate; GDP, guanosine 5′-diphosphate; GTPase, guanosine-5′-triphosphatase, EC 3.6.1; tRNA, transfer ribonucleic acid; $tRNA^{Arg}$ and $tRNA^{Phe}$, purified arginine- and phenylalanine-accepting tRNA, respectively; $tRNA_1^{Val}$, purified isoacceptor 1 of valine-accepting tRNA; aa-tRNA, aminoacyl-tRNA; Arg-$tRNA^{Arg}$, Phe-$tRNA^{Phe}$, and Val-$tRNA_1^{Val}$, purified specific tRNA charged with Arg, Phe, and Val, respectively; C-C-A, uncharged 3′-terminal trinucleoside diphosphate; C-C-A-aa, 3′-aminoacyl-C-C-A; C-C-A-Val, 3′-terminal trinucleoside diphosphate charged with Val; EDTA, ethylenediaminetetraacetic acid; DTT, 1,4-dithiothreitol; PEP, phosphoenolpyruvate.

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