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Biochimica et Biophysica Acta 1412 (1999) 66–77

BIOCHIMICA ET BIOPHYSICA ACTA
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The plasma membrane NADH oxidase of HeLa cells has hydroquinone oxidase activity

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Received 1 February 1999; received in revised form 15 March 1999; accepted 16 March 1999

Abstract

The plasma membrane NADH oxidase activity partially purified from the surface of HeLa cells exhibited hydroquinone oxidase activity. The preparations completely lacked NADH:ubiquinone reductase activity. However, in the absence of NADH, reduced coenzyme Q_{10} ($Q_{10}H_2$ = ubiquinol) was oxidized at a rate of 15 ± 6 nmol min^{-1} mg $protein^{-1}$ depending on degree of purification. The apparent K_m for $Q_{10}H_2$ oxidation was 33 μM. Activities were inhibited competitively by the cancer cell-specific NADH oxidase inhibitors, capsaicin and the antitumor sulfonylurea *N*-(4-methylphenylsulfonyl)-*N′*-(4-chlorophenyl)urea (LY181984). With coenzyme Q_0, where the preparations were unable to carry out either NADH:quinone reduction or reduced quinone oxidation, quinol oxidation was observed with an equal mixture of the Q_0 and Q_0H_2 forms. With the mixture, a rate of Q_0H_2 oxidation of 8–17 nmol min^{-1} mg $protein^{-1}$ was observed with an apparent K_m of 0.22 mM. The rate of $Q_{10}H_2$ oxidation was not stimulated by addition of equal amounts of Q_{10} and $Q_{10}H_2$. However, addition of Q_0 to the $Q_{10}H_2$ did stimulate. The oxidation of $Q_{10}H_2$ proceeded with what appeared to be a two-electron transfer. The oxidation of Q_0H_2 may involve Q_0, but the mechanism was not clear. The findings suggest the potential participation of the plasma membrane NADH oxidase as a terminal oxidase of plasma membrane electron transport from cytosolic NAD(P)H via naturally occurring hydroquinones to acceptors at the cell surface.

Keywords: NADH oxidase; Hydroquinone oxidase; Coenzyme Q; Ubiquinone; Diarylsulfonylurea; Capsaicin; Antitumor sulfonylurea; HeLa cell

1. Introduction

Coenzyme Q or ubiquinone (Q) [1] is an essential lipid-soluble electron transport component widely distributed among various subcellular locations including the plasma membrane [2]. At the plasma membrane, reduced ubiquinone (QH_2) may function primarily as an antioxidant by inhibiting lipid peroxidation or protein carbonylation [3,4]. Alternatively, it may participate in the plasma membrane electron transport chain and growth control [5].

Recently, Morré and coworkers described a

Abbreviations: Q, coenzyme Q; Q_{10}, coenzyme Q_{10} or ubiquinone = 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone; QH_2, coenzyme Q hydroquinone; $Q_{10}H_2$, reduced coenzyme Q_{10} or ubiquinol = 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinol; Q_0, coenzyme Q_0, 2,3-dimethoxy-5-methyl-1,4-benzoquinone; Q_0H_2, reduced coenzyme Q_0, 2,3-dimethoxy-5-methyl-1,4-benzoquinol; capsaicin, 8-methyl-*N*-vanillyl-6-noneamide; LY181984, *N*-(4-methylphenylsulfonyl-*N′*-(4-chlorophenyl) urea; LY181985, *N*-(4-methylphenylsulfonyl-*N′*-(4-phenyl) urea

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0005-2728/99/$ – see front matter
PII: S0005-2728(99)00049-3

plasma membrane NADH oxidase of HeLa and other transformed cells where activity was inhibited by several quinone site inhibitors including the vanilloid capsaicin [6] and the antitumor sulfonylurea *N*-(4-methylphenylsulfonyl)-*N'*-(4-chlorophenyl)urea (LY181984) [7]. The catalytic activity of the protein was resistant to heating and to protease digestion [8] and could be released from HeLa cells by treatment at pH 5 [9]. Preparation of partially purified NADH oxidase released from HeLa cells by pH 5 treatment followed by heat (50°C, 10 min) and proteinase K [9] lacked NADH:ubiquinone reductase activity. However, the present paper demonstrates an ability of these preparations to oxidize $Q_{10}H_2$ that parallels the ability of these preparations to oxidize NADH. These observations have implications in mechanisms to protect plasma membranes against reactive oxygen species and lend support to previous suggestions that the plasma membrane NADH oxidase under certain conditions may function as a terminal oxidase of a plasma membrane electron transport chain that transfers electrons from cytosolic NAD(P)H via QH_2 to molecular oxygen or to protein disulfides as terminal electron acceptors [10,11].

2. Materials and methods

2.1. Materials

Coenzyme Q_{10} (2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone, Lot 92040702) was kindly donated by Eisai, Bunkyou-ku, Tokyo, Japan. Coenzyme Q_0 (2,3-dimethoxy-5-methyl-1,4-benzoquinone, Lot 68F0802) was from Sigma, St. Louis, MO.

The method for preparation of reduced coenzyme Qs (50 mM coenzyme Q_0 stock solution or 7.5 mM coenzyme Q_{10} stock solution in ethanol) involved addition of an equal volume of 0.25% $NaBH_4$ under nitrogen followed after several min by 0.1 vol. of 0.1 N HCl to degrade the excess $NaBH_4$. The colorless solution was held at room temperature and prepared fresh for each experiment.

2.2. Preparation of HeLa cells and cell-free extracts

HeLa S cells were collected by centrifugation and shipped frozen in 0.1 M sodium acetate, pH 5, in a ratio of 1 ml packed cell volume to 1 ml of acetate (Cellex Biosciences, Minneapolis, MN). The cells were thawed at room temperature, resuspended and incubated at 37°C for 1 h to complete release of the protein. The cells were removed by centrifugation at $37\,000\times g$, for 60 min and the cell-free supernatants were refrozen and stored in 1-ml aliquots at −70°C.

For heat treatment, 1-ml aliquots of the above supernatant material were thawed at room temperature and heated to 50°C for 10 min. The denatured proteins were removed by centrifugation ($1500\times g$, 5 min, in 1-ml aliquots). Full NADH oxidase activity was retained from this step (Table 1).

For protease treatment, the pH of the heat-stable supernatant was adjusted to 7.8 by addition of 0.1 M sodium hydroxide. *Tritirachium album* proteinase K (Calbiochem) was added (4 ng ml^{-1}) and incubated at 37°C for 1 h with full retention of enzymatic activity and drug response (Table 1, [9]). The reaction was stopped either by freezing for determination of enzymatic activity or by addition of 0.1 M phenylmethylsulfonyl fluoride (PMSF) in ethanol to yield a final concentration of 10 mM PMSF. Activity was measured by oxidation of NADH (decrease in absorbance at 340 nm) and inhibition of NADH oxidation by capsaicin or LY181984.

The above purification protocol has been employed to purify the drug-responsive NADH oxidase both from human sera [8] and from the surface of HeLa cells [9] without loss of drug-responsive NADH oxidase activity. Resistance of the 34-kDa cell surface NADH oxidase of the HeLa was confirmed by Western blot analysis (Fig. 1; see also Figs. 4 and 6 of reference [12]). The bulk of the proteins other than the NADH oxidase that are released from HeLa cells, including low levels of cytosolic dehydrogenases that may be released from leaky or broken cells, do not survive the heat and proteinase K treatments.

2.3. Enzyme assay for NADH-Q reductase

The assay for NADH-Q reductase was in 50 mM Tris-HCl, pH 7.2, containing 200 μM Q_0, 0.05–0.1 mg protein in a final volume of 2.5 ml. Q reduction was monitored by the decrease in absorbance at 410 nm as described by Sun et al. [5] using a Hitachi U3210 spectrophotometer for two consecutive 5-min

periods. The decrease in absorbance during the second 5-min period was used to calculate the specific activity. A blank rate was subtracted in which the assay was carried out in the absence of added proteins. The extinction coefficient used for Q reduction was 0.805 mM^{-1} cm^{-1} [1,5].

2.4. Enzyme assay for reduced coenzyme Q oxidase

For oxidation of Q_0H_2 the reaction mixture contained indicated amounts of HeLa cell surface protein in 2.5 ml of 50 mM Tris-MES buffer, pH 7.0 with or without 30 μl of 100 mM EDTA and the reaction was started by the addition of 40 μl of 25 mM Q_0H_2 or 40 μl of an equal mixture of Q_0H_2 and Q_0 (25 mM) as indicated. The Q_0H_2 oxidase activity was measured spectrophotometrically at 410 nm at 37°C.

For oxidation of $Q_{10}H_2$, 0.08% Triton X-100 was used to solubilize the $Q_{10}H_2$ in the assay buffer. The reaction was started with the addition of 40 μl of 5 mM $Q_{10}H_2$. The oxidase activity was measured by following the increase in absorbance at 410 nm. With $Q_{10}H_2$+Q_0 mixtures, 2.5 mM Q_0, was added to start the reaction.

Rates were determined by continuous recording over two periods of 5 min each. Rates were calculated from the second, steady-state, 5-min period (e.g. Figs. 2A and 3A). An extinction coefficient of 0.805 mM^{-1} cm^{-1} was used to calculate the rate of Q reduction [5].

Q_{10} in ethanol exhibits a maximum at 275 nm, a broad band at about 410 nm and a minimum at 236 nm [14]. Upon reduction of the quinone, the bands at 275 and 410 nm disappear and a new peak characteristic of $Q_{10}H_2$ appears at 290 nm [1]. In order to correlate the NADH oxidase and hydroquinone oxidase activities, assays were carried out in

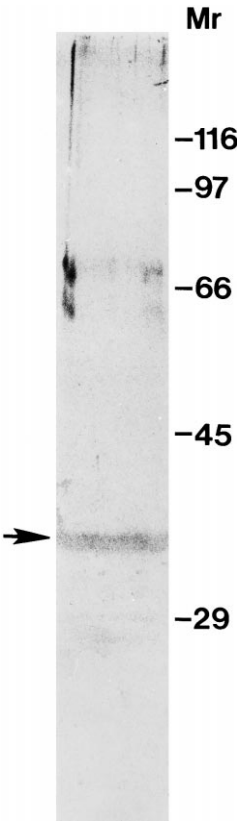

Fig. 1. Western blot analysis with monospecific rabbit antisera as described [13] after 10% polyacrylamide gel electrophoresis illustrating the presence of p34 NOX (arrow) in the fraction containing the capsaicin-inhibited NADH oxidase released from HeLa cells by pH 5 treatment and following heat and proteinase K treatment. The immunoreactive protein was present almost exclusively as the 34-kDa monomer. Molecular mass is indicated in kDa.

Table 1
Recovery of protein and NOX activity from HeLa S cells grown in suspension culture, release by low pH treatment, resistance to heating and proteinase K and response to 1 μM capsaicin (Cap)

Fraction	Total protein (mg)	Total activity (nmol min^{-1})	Specific activity (nmol min^{-1} mg $protein^{-1}$)	
			No Cap	1 μM Cap
HeLa S cells	1000	200	0.20	0.14
pH 4.5, 37°C, 60 min supernatant	20	160	8.0	5.3
50°C, 20 min supernatant	16	253	16.0	10.6
50°C, 20 min pellet	4	–[a]	–	–
Proteinase K treatment of 50°C supernatant	11	204	18.5	11.2

[a]No activity.

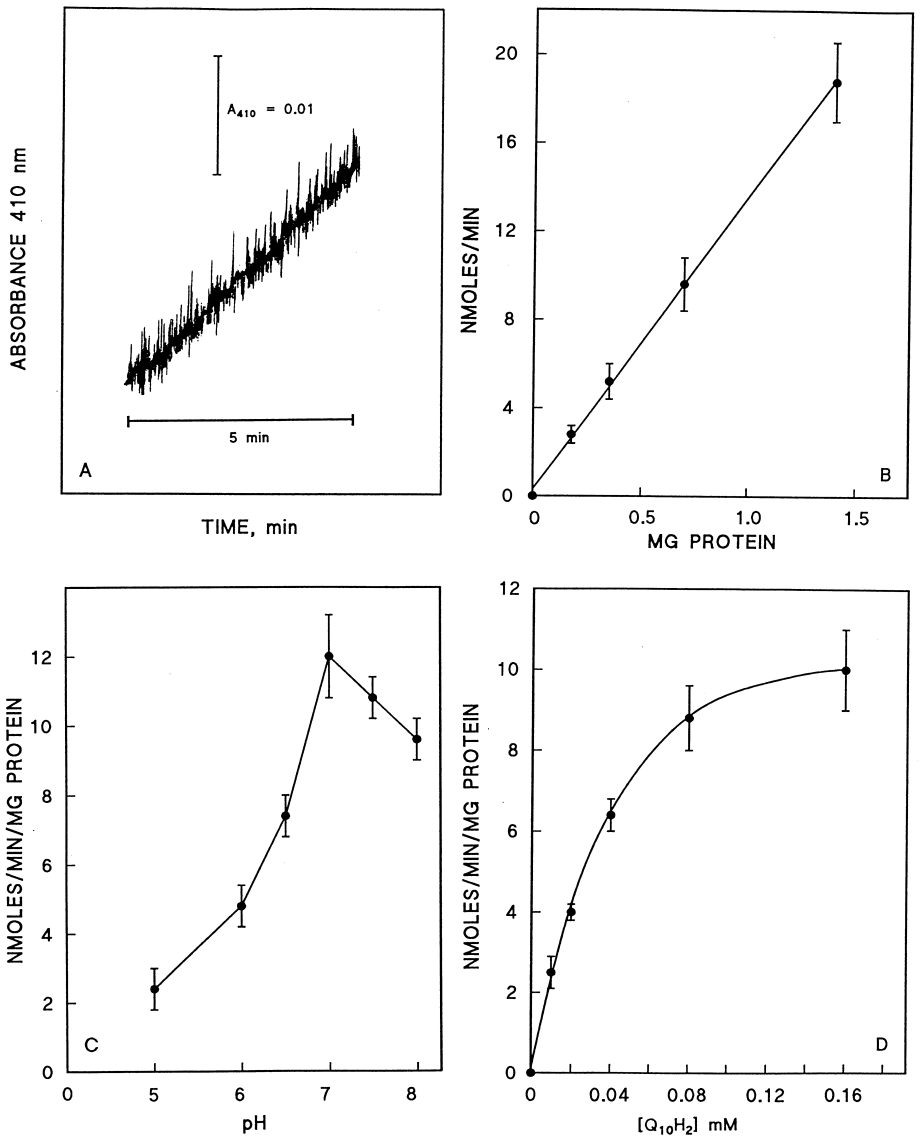

Fig. 2. Oxidation of $Q_{10}H_2$ by an enzymatic preparation with NADH oxidase activity solubilized from HeLa cells by pH 5 treatment, followed by heat and proteinase K. The disappearance of $Q_{10}H_2$ was determined from the increase in absorbance at 410 nm. (A) Increase in absorbance with 1 mg protein at 410 nm as a function of time. (B) Rate of $Q_{10}H_2$ oxidation in the presence of 160 μM $Q_{10}H_2$ in proportion to amount of added protein. In the presence of 80 μM $Q_{10}H_2$ proportionality was achieved up to 0.6 mg protein (not shown). (C) Response to pH. (D) Saturation kinetics with increasing amounts of $Q_{10}H_2$ in the assay. Unless specified otherwise, the concentration of $Q_{10}H_2$ was 80 μM, the pH was 7 and the protein was 0.3 mg. Assays were at 37°C for two intervals of 5 min each. Rates were based on the slope of the second 5-min assay.

the presence of compounds, such as capsaicin, that absorb in the range 250–300 nm. Therefore, the method of Sun et al. [5] which measures Q oxidation and reduction at 410 nm was employed.

2.5. Differential spectra of coenzyme Qs

The spectral changes of ubiquinol during the enzyme reaction were measured against the reaction mixture without ubiquinol in the reference cell.

3. Results

The quinol oxidase activity was assayed with solubilized, partially purified preparations containing the

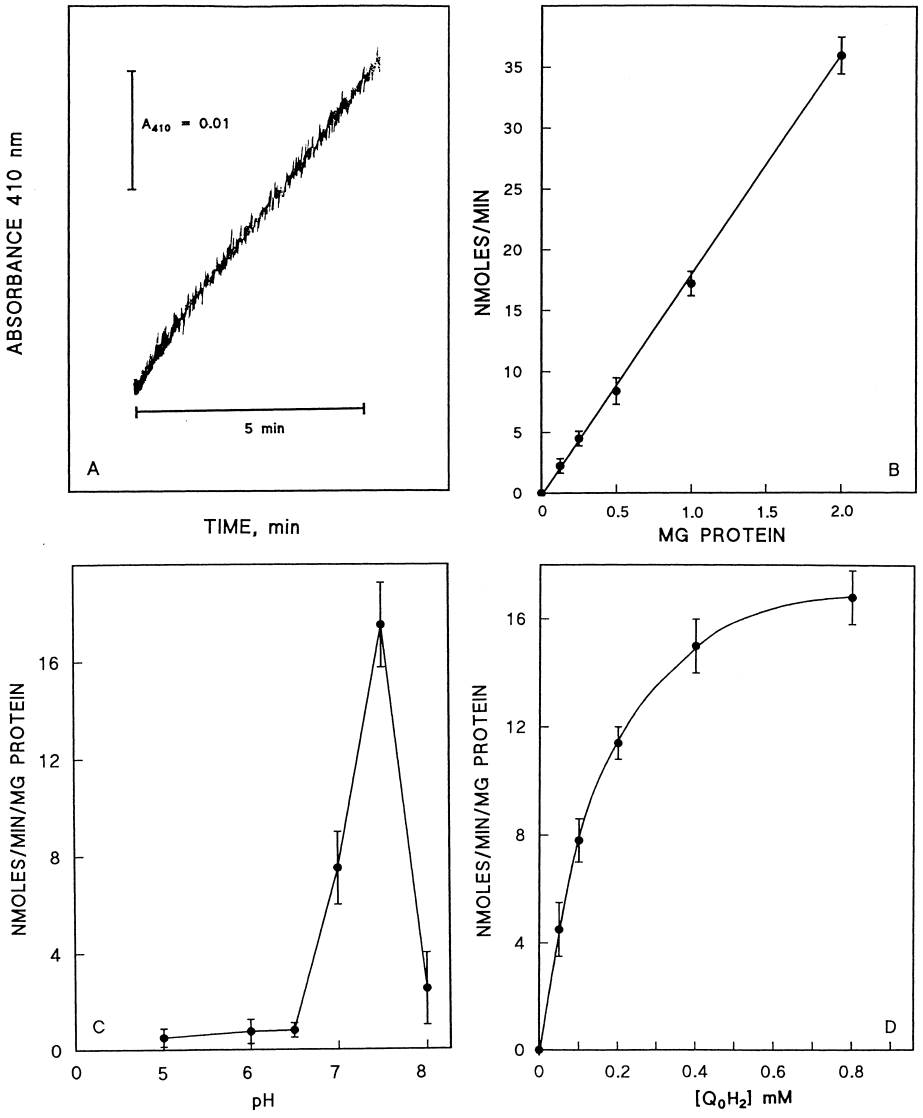

Fig. 3. Oxidation of Q_0H_2 (the substrate was a 50:50 mixture of Q_0 and Q_0H_2) by an enzymatic preparation with NADH oxidase activity solubilized from HeLa cells by treatment at pH 5, followed by 20 min at 50°C and incubation with proteinase K as described in Section 2. The disappearance of Q_0H_2 was determined from the increase in absorbance at 410 nm. (A) Increase in absorbance at 410 nm as a function of time (1 mg protein). (B) Rate of Q_0H_2 oxidation in proportion to amount of added protein at a concentration of Q_0+Q_0H_2 of 0.8 mM. At a concentration of Q_0+Q_0H_2 of 0.4 mM, proportionality was observed up to 1.5 mg protein (not shown). (C) Response to pH. (D) Saturation kinetics with increasing amounts of Q_0H_2 in the assay. Unless specified otherwise, the concentration of Q_0+Q_0H_2 was 0.4 mM, the pH was 7 and the protein concentration was 0.3 mg protein per assay. Assays were at 37°C for two intervals of 5 min each. Rates were based on the slope of the second 5-min assay.

NADH oxidase activity released from the HeLa cell surface. Plasma membranes also contained a vigorous NADH:quinone reductase, unrelated to the NADH oxidase, that generated both Q_0H_2 and $Q_{10}H_2$ at the expense of NADH. The NADH oxidase preparations analyzed here completely lacked NADH:quinone reductase activity. Neither Q_0 nor Q_{10} was reduced in the presence of NADH by these preparations under conditions where QH_2 oxidation was observed.

In contrast to Q_{10} where no $Q_{10}H_2$ formation was observed either in the presence or absence of NADH, the NADH oxidase preparations solubilized from the HeLa cell surface were capable of $Q_{10}H_2$ oxidation.

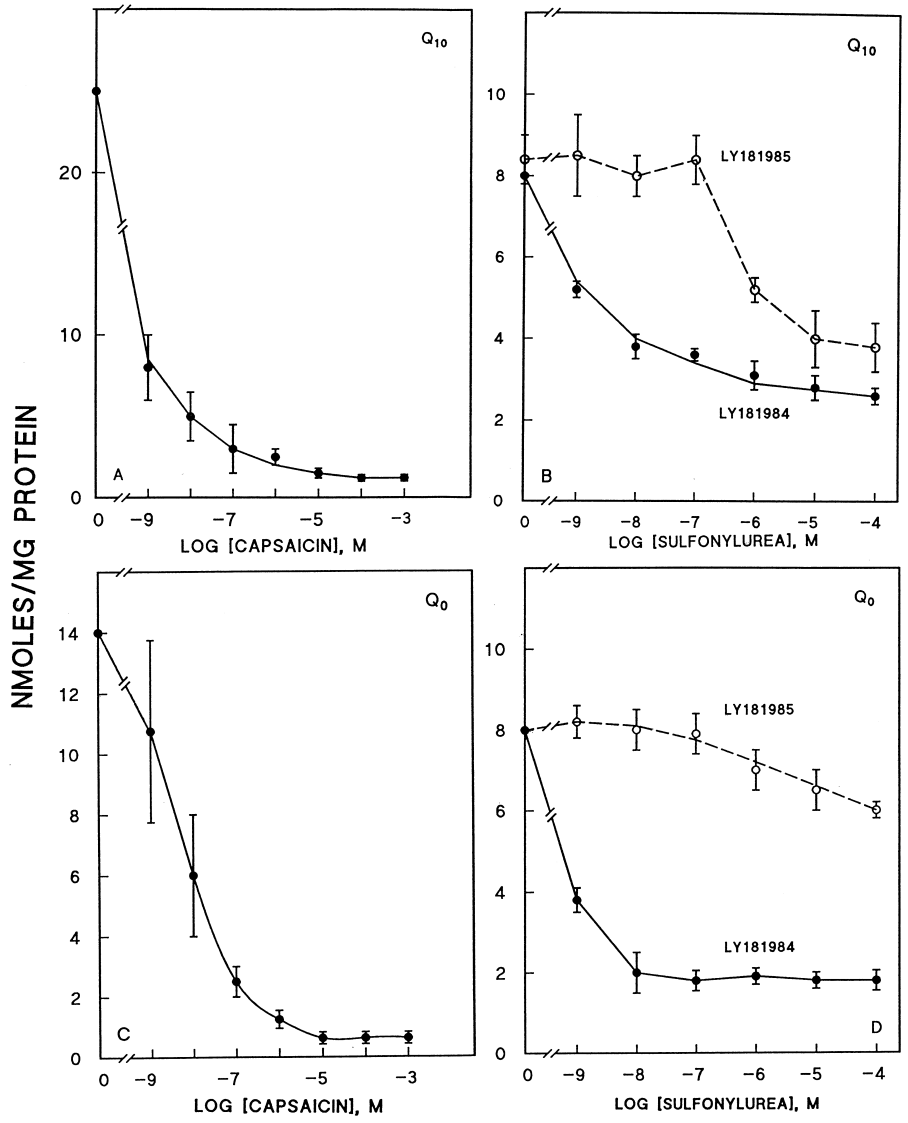

Fig. 4. Response of oxidation of $Q_{10}H_2$ to the vanilloid capsaicin (A) and to the active (LY181984) and the inactive (LY181985) antitumor sulfonylureas (B) and the response of oxidation of Q_0H_2 to the vanilloid capsaicin (C) and to the active (LY181984) and the inactive (LY181985) antitumor sulfonylureas (D). For $Q_{10}H_2$, the assay conditions were as described in Fig. 2. For Q_0H_2, the assay conditions were as described in Fig. 3.

The specific activity was 15 ± 6 nmol min^{-1} mg $protein^{-1}$ and of the same order of magnitude as the oxidation of NADH.

The oxidation of $Q_{10}H_2$ was proportional to time (Fig. 2A) and protein concentration (Fig. 2B). The pH optimum for oxidation of $Q_{10}H_2$ was 7.0 (Fig. 2C). The activity exhibited saturation kinetics with respect to substrate (Fig. 2D). The apparent K_m for $Q_{10}H_2$ was 33 ± 12 μM (Table 2). After initial equilibration, absorbance changes at 410 nm in the absence of $Q_{10}H_2$ were negligible. Oxidation of $Q_{10}H_2$ by the HeLa preparations was unaffected by either superoxide dismutase or catalase. The rates of spontaneous $Q_{10}H_2$ oxidation were too low to determine accurately (cf. Fig. 6), but were estimated to be less than 0.1 the enzyme-catalyzed rate at a substrate concentration of 80 μM. Oxygen consumption measured in parallel using an oxygen electrode was 2.5 ± 0.25 nmol min^{-1} mg $protein^{-1}$ suggesting substantial transfer of electrons to protein thiols as an alternative electron acceptor as described previously for NADH oxidation [15,16].

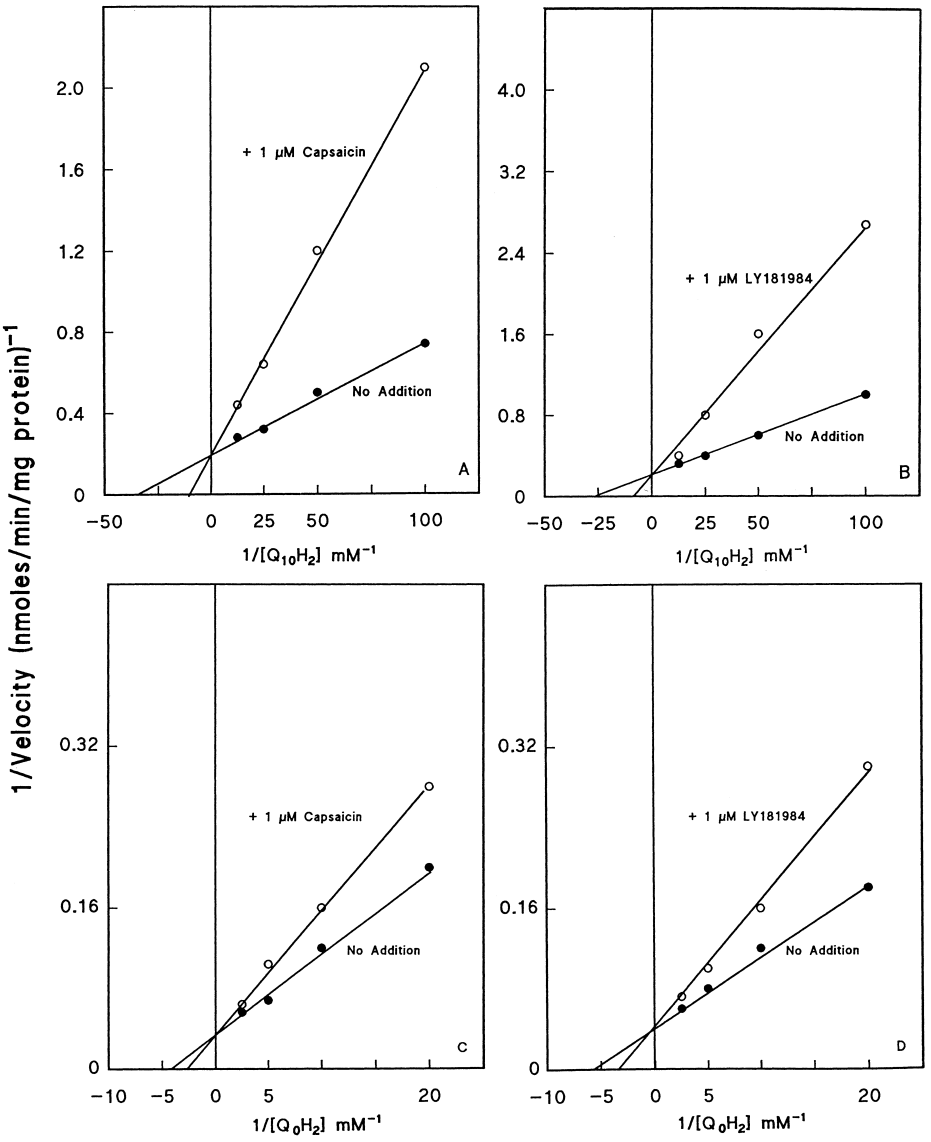

Fig. 5. Lineweaver–Burke analyses of the inhibition of oxidation of $Q_{10}H_2$ (A,B) or Q_0H_2 (C,D) by 1 μM capsaicin (A,C) and by 1 μM LY181984 (B,D). Plotted are the double reciprocal plots of 1/reaction rate in nmol min^{-1} mg protein^{-1} vs. 1/[reduced coenzyme].

The fraction with NADH oxidase activity released from the HeLa cell surface was without effect either on the reduction of Q_0 in the presence of NADH or on the oxidation of $Q_{10}H_2$ (no activity). However, with an equal mixture of 0.2 mM Q_0 and 0.2 mM Q_0H_2, the Q_0H_2 was oxidized (Fig. 3) with a specific activity of 8–17 nmol min^{-1} mg protein^{-1}.

As with the oxidation of $Q_{10}H_2$, the oxidation of the Q_0H_2 was proportional to time (Fig. 3A) and to protein concentration (Fig. 3B). The pH optimum was 7.5 (Fig. 3C). The rate was proportional to concentration of total Q_0 (reduced plus oxidized) (Fig. 3D) with an apparent K_m of 220 ± 70 μM (Table 2).

The blank rates for Q_0H_2 oxidation were estimated to be less than 0.2 the enzyme-catalyzed rates. Rates of oxygen consumption with the mixtures of oxidized and reduced coenzyme Q_0 using an oxygen electrode were 3.75 ± 0.4 nmol min^{-1} mg protein^{-1}.

Both the oxidation of $Q_{10}H_2$ and the equal mixture of the Q_0 and Q_0H_2 were resistant to KCN (1 mM), to heating at 50°C for 15 min and to digestion

Table 2
Kinetic constants of hydroquinone oxidation by the drug-responsive NADH oxidase-enriched fraction released from HeLa cells by low pH treatment

Hydroquinone	Apparent K_m (mM)	K_I (μM)	
	No addition	1 μM Capsaicin	1 μM LY181984
Q_0H_2	0.220 ± 0.070 (n = 4)	2.0	1.2
$Q_{10}H_2$	0.033 ± 0.012 (n = 5)	0.7 (n = 2)	0.3 (n = 2)

with proteinase K as was NADH oxidation by the HeLa cell preparations solubilized by pH 5 treatment. Evidence for the identity of the two activities was provided by parallel neutralization of both activities by a specific antibody, inhibitor studies, and the functional periodicity of the activity. Using a monoclonal antibody generated to the serum form of the activity from cancer patients [8], the $Q_{10}H_2$ oxidase activity was inhibited to basal levels at an antibody dilution of 1:5000. A similar inhibition of activity (40%) was achieved with the 1:1 mixture of Q_0 and Q_0H_2. These same antisera recognized the 34-kDa form of the oxidase in the HeLa cell preparations following heat treatment and proteinase K digestion (Fig. 1). After proteinase K digestion, the proteins present were largely fragments of MW $< 15\,000$ kDa. The NOX protein was among the few high molecular weight proteins that survived the proteinase K digestion [8,9]. Other NADH dehydrogenase and oxidase activities including contaminating cytosolic enzymes rapidly lost activity in the presence of proteinase K.

Two well-characterized inhibitors of the HeLa cell surface NADH oxidase inhibited the ubiquinol oxi-

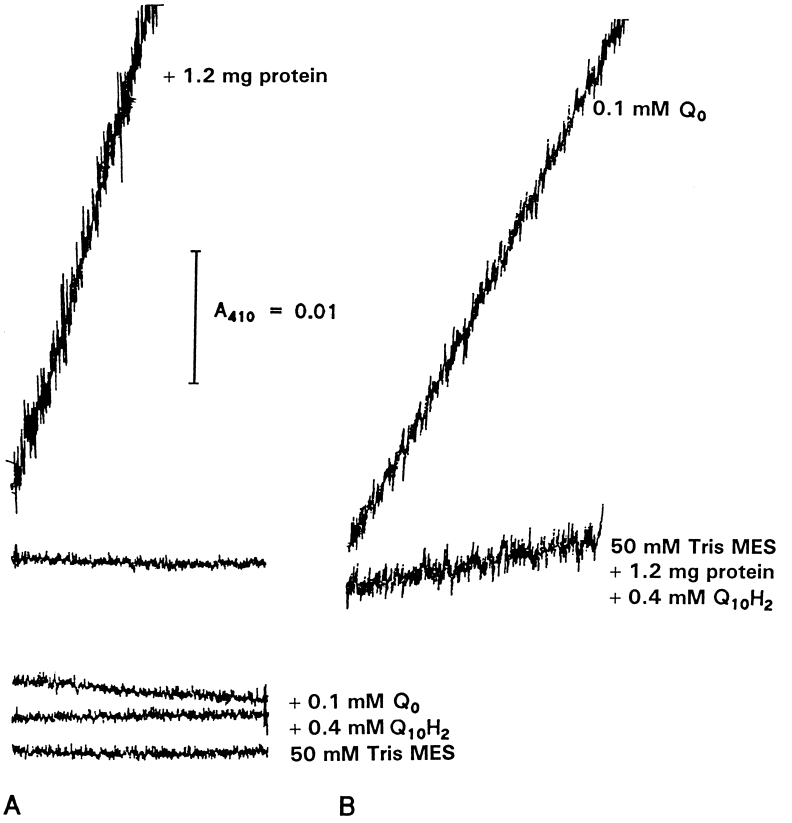

Fig. 6. Reaction rates of oxidation of 0.2 mM $Q_{10}H_2$ stimulated by the addition of 0.2 mM Q_0. A 1:1 ratio of $Q_{10}H_2$ to Q_0 gave optimal activity. (A) The enzyme source added last to the complete mixture. (B) The Q_0 added last to the complete mixture.

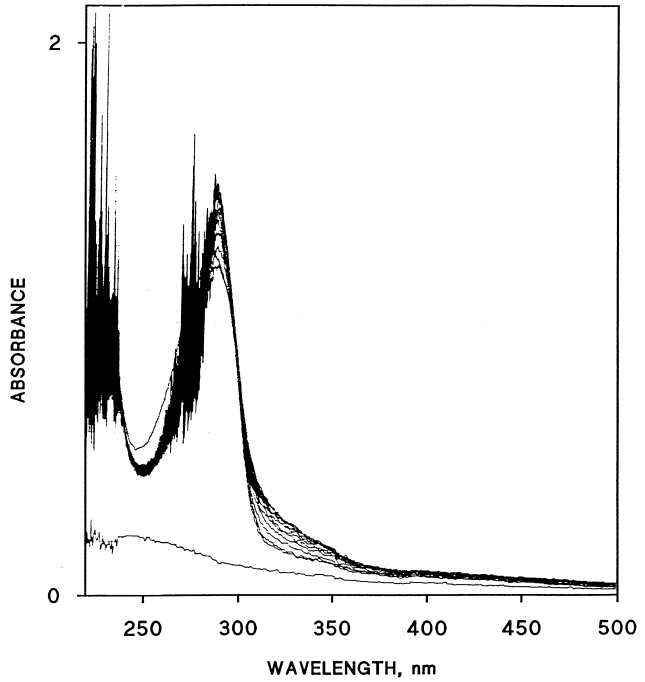

Fig. 7. Oxidation of $Q_{10}H_2$ (0.2 mM) in the presence of 0.2 mM Q_0. The preparation contained 0.6 mg protein, 1.2 mM EDTA, 0.08% Triton X-100 and 50 mM Tris-MES buffer, pH 7.0. The preparation was scanned every 5 min for 50 min. Oxidation of $Q_{10}H_2$ is indicated by the increase in absorbance at 410 nm accompanied by a decrease in absorbance at 290 nm.

dase as well. The inhibition was half maximal in the nanomolar or subnanomolar range of concentrations for both capsaicin (Fig. 4A) and the antitumor sulfonylurea *N*-(4-methylphenylsulfonyl)-*N*′-(4-chlorophenyl)urea (LY181984) (Fig. 4B). With the oxidation of Q_0H_2 (as the mixture of Q_0 and Q_0H_2) activity also was inhibited by both capsaicin (Fig. 4C) and by the antitumor sulfonylurea (LY181984) (Fig. 4D) with approximately the same concentration dependence as for oxidation of $Q_{10}H_2$. When analyzed from Lineweaver–Burke double reciprocal plots, the inhibitions by both capsaicin and LY181984 were found to be competitive as shown by the convergence to the same ordinate intercept (same apparent V_{max}) and the characteristic shift of the intersection with the *x*-axis due to competition of enzyme and substrate for the same binding site (Fig. 5).

Whereas Q_{10} was without effect on $Q_{10}H_2$ oxidation, $Q_{10}H_2$ oxidation was stimulated by Q_0 (Fig. 6). Under conditions where a steady-state rate of $Q_{10}H_2$ oxidation was observed, the rate of $Q_{10}H_2$ oxidation was stimulated by the addition of Q_0. An equivalent response was obtained either by adding the enzyme source first (Fig. 6A) or last (Fig. 6B) to the complete reaction mixture.

A stoichiometric reduction of Q_0 at the expense of $Q_{10}H_2$ did not appear to be the basis for the stimulated activity. A stoichiometric reduction of one quinone at the expense of the other would have resulted in no net change in optical density since the extinction coefficients of Q_0 and Q_{10} at 410 nm were similar. However, as shown by the change in the absorbance spectra of Fig. 7, a net oxidation of $Q_{10}H_2$ did occur in the presence of Q_0.

A property of the NADH oxidase is that its activity fluctuates with a period of about 24 min (see Section 4). The activity of the hydroquinone oxidase also was periodic (Fig. 8). The activity cycled through four periods in 94.5 min with an average period of 23.6 min. This periodicity, which is characteristic of the NADH oxidase activity as well, provides additional evidence that the hydroquinone oxidase and NADH oxidase activities are catalyzed by the same protein.

4. Discussion

In 1986, our laboratory first described from plants an NADH oxidase activity that was growth factor stimulated [17]. This activity was purified from a plant source [18], shown to be present in mammalian plasma membranes where it was also hormone and growth factor stimulated [19], and was subsequently shown to be associated with the external plasma membrane surface [20]. Since it is unlikely that external concentrations of NADH would ever reach 1 mM, an alternative function was sought in protein disulfide–thiol interchange [16,21,22]. Activity was shown by measurement of restoration of activity to scrambled (inactive) RNase [21,22] and from an overall stimulation of the reduction of protein disulfides in the presence of NADH (NADH:protein disulfide reductase) and other reducing agents [16]. Both molecular oxygen [15] and protein disulfides [15,16] apparently serve as electron acceptors for electron transfer from NADH. However, evidence for a physiological electron donor other than thiols was unavailable. In this report, we show that $Q_{10}H_2$

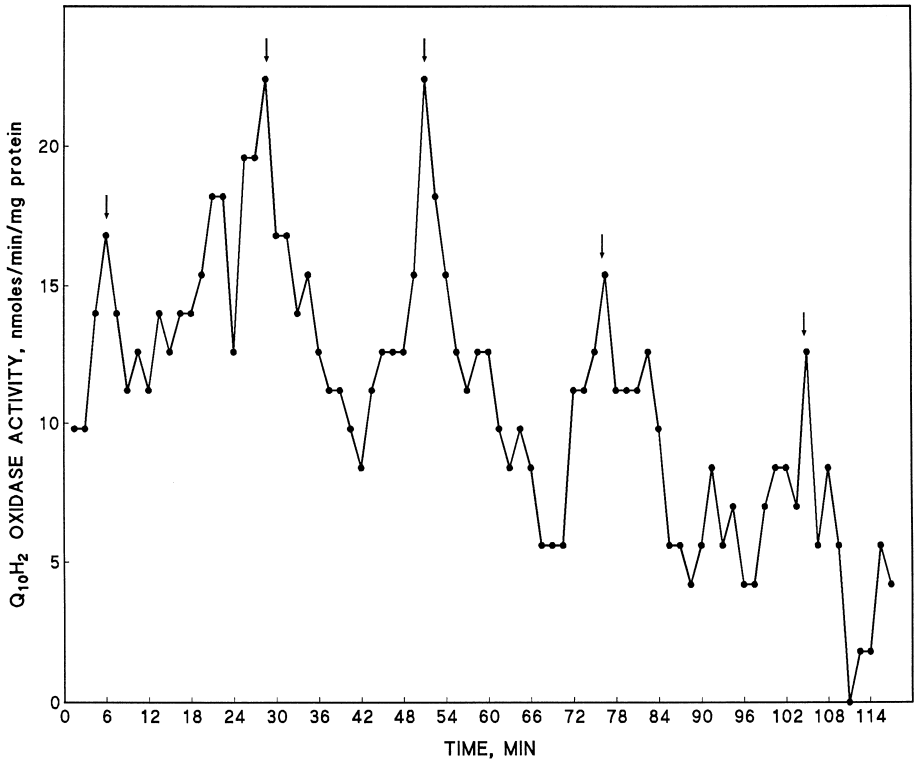

Fig. 8. Periodic variation in the rate of oxidation of $Q_{10}H_2$ as a function of time over 114 min showing five maxima (6, 28.5, 52.5, 76.5 and 100.5 min). Within a time of 94.5 min, four maxima were observed with an average period length of 23.6 ± 0.6 min per period. By 100 min, 60% of the substrate had been consumed and the decline in reaction rate during the fourth full cycle is attributed to substrate depletion. A similar variation in oxidation rate of NADH was observed with these preparations. The enzyme source was the solubilized and partially purified NOX preparation from the HeLa cell surface. Rates were determined over 1 min every 1.5 min. Conditions were as given for Fig. 2.

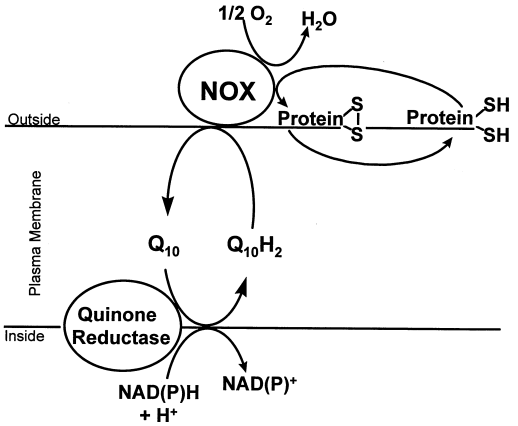

Fig. 9. Diagram illustrating the spatial relationships across the plasma membrane of the inside NAD(P)H:quinone reductase, the membrane pool of coenzyme Qs and the external NADH oxidase (NOX) protein. As illustrated, the NOX protein could function as a terminal oxidase of plasma membrane electron transport donating electrons from cytosolic NADH either to molecular oxygen in a two-electron transfer or to reduce protein disulfides.

or Q_0H_2 can serve as electron donors for oxygen or protein disulfide reduction catalyzed by the plasma membrane located NADH oxidase protein with protein disulfide–thiol interchange activity (NOX).

It is well known that plasma membranes contain high concentrations of quinones including Q_9 [2]. These quinones become reduced at the inner surface of the plasma membrane through the activity of an NAD(P)H coenzyme Q reductase or diaphorase at the plasma membrane [4]. A similar activity is located in the cytosol [23]. The QH_2 would be free to migrate through the lipid interior of the membrane where it would become available for oxidation by the NOX protein (Fig. 9). To our knowledge, this is the first formulation of a plasma membrane electron transport chain based on identification of known proteins and constituents in the plasma membrane and involving physiological electron donors and acceptors.

Crane et al. [24] had previously described electron

transport chains in the plasma membrane based on reduction of ferricyanide [25] which is not a physiological electron acceptor or to iron bound to diferric transferrin. The NOX protein is distinct from the NADH-ferricyanide reductase [26] and shows no activity with ferricyanide or diferric transferrin as an electron acceptor. Both ferricyanide and diferric transferrin exhibit growth factor activity and do stimulate the NOX activity of rat liver plasma membrane as growth factors [27,28], but not as electron acceptors.

The specific activity of hydroquinone oxidation, while low, ca. 15 nmol min^{-1} mg $protein^{-1}$, is of the same order of magnitude as measured for the transmembrane flow of electrons across HL60 [29] and HeLa cells. Additionally, the oxidation of hydroquinones is inhibited competitively by two inhibitors of the plasma membrane NADH oxidase, capsaicin [6] and the antitumor sulfonylurea, LY181984 [7], suggesting that these compounds act at the same site and that the two activities reside on the same protein. These observations certainly do not prove a role of the NOX protein in transmembrane electron transport, but do now raise the possibility. The NOX protein might also function in the scavenging of active oxygen forms at the plasma membrane and, as suggested by Roualt and Klausner [30], possibly in oxygen sensing.

We have suggested previously that a second important function of the NOX protein is that of protein disulfide–thiol interchange in a growth-related process of breakage and reformation of disulfide bonds [15]. Transfer of electrons from NADH or NADPH via hydroquinones in the membrane might have important implications in keeping plasma membrane proteins reduced, in sensing of the redox state of the cytoplasm in relation to growth control, or as a possible mechanism of linking sensing systems at the internal cell surface to redox changes at the external cell surface as have been postulated to occur in aging [31–33].

Oxygen consumption measured with an oxygen electrode was less than QH_2 oxidation. A similar lack of stoichiometry between NADH oxidation and O_2 consumption was observed with the NADH oxidase and shown to result from reduction of protein disulfides as alternate electron acceptors to oxygen [16]. Up to 50% or more of the electrons coming from NADH appear to be transferred to protein disulfides. A similar explanation may account for the lack of stoichiometry between oxygen consumption and hydroquinone oxidation reported here.

A recently described property of the NADH oxidase is its periodicity [34,35]. The activity oscillates to a maximum with a regular period of about 24 min. The basis for and the significance of the periodicity is unknown, but a potential time-keeping function is indicated [34,35]. That the oxidation of $Q_{10}H_2$ and the oxidation of NADH [35] both show a similar 24-min periodicity support our suggestion that the two activities may be attributed to the same protein or protein complex.

Acknowledgements

We thank Andrew Bridge for his assistance with assays.

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