Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4887–4891, May 1995
Biochemistry

Coenzyme Q reductase from liver plasma membrane: Purification and role in trans-plasma-membrane electron transport

JOSÉ M. VILLALBA, FRANCISCO NAVARRO, FRANCISCO CÓRDOBA*, ANTONIO SERRANO, ANTONIO ARROYO, FREDERICK L. CRANE†, AND PLÁCIDO NAVAS‡

Departamento de Biología Celular, Facultad de Ciencias, Universidad de Córdoba, 14004 Córdoba, Spain

Communicated by Karl Folkers, University of Texas, Austin, TX, January 25, 1995 (received for review November 17, 1994)

ABSTRACT **A specific requirement for coenzyme Q in the maintenance of trans-plasma-membrane redox activity is demonstrated. Extraction of coenzyme Q from membranes resulted in inhibition of NADH-ascorbate free radical reductase (trans electron transport), and addition of coenzyme Q_{10} restored the activity. NADH-cytochrome *c* oxidoreductase (cis electron transport) did not respond to the coenzyme Q status. Quinone analogs inhibited trans-plasma-membrane redox activity, and the inhibition was reversed by coenzyme Q. A 34-kDa coenzyme Q reductase (p34) has been purified from pig-liver plasma membranes. The isolated enzyme was sensitive to quinone-site inhibitors. p34 catalyzed the NADH-dependent reduction of coenzyme Q_{10} after reconstitution in phospholipid liposomes. When plasma membranes were supplemented with extra p34, NADH-ascorbate free radical reductase was activated but NADH-cytochrome *c* oxidoreductase was not. These results support the involvement of p34 as a source of electrons for the trans-plasma-membrane redox system oxidizing NADH and support coenzyme Q as an intermediate electron carrier between NADH and the external acceptor ascorbate free radical.**

Coenzyme Q (CoQ, ubiquinone) is a lipophilic redox compound that is present in all animal-cell membranes (1). In the inner mitochondrial membrane, CoQ plays a key role shuttling electrons from NADH and succinate dehydrogenases to the cytochrome b–c_1 complex. The role of CoQ in extramitochondrial membranes is not as well characterized, and functions such as storage for transport to mitochondria or the blood (1) or protection of unsaturated membrane lipids from oxidative damage (2) have been proposed.

It has recently been demonstrated that CoQ can function as an intermediate electron carrier in plasma membrane (PM)-associated electron transport regulating cell growth (3), but no distinction between cis and trans electron transport was made. Extraction of CoQ from PMs inhibits NADH-ferricyanide (FeCN) oxidoreductase and ferric transferrin-stimulated NADH oxidase, and addition of CoQ restores redox activities. Also, FeCN reduction by PMs is inhibited by CoQ analogs, and addition of CoQ partially restores the activity (3).

The existence of a significant amount of CoQ in the quinol state in the PM (4) indicates the necessity of a system for reduction of intramembrane CoQ. Reduction of CoQ in the PM upon addition of NADH has been detected at 410 nm (3). However, no proteins involved in the NADH-dependent CoQ reduction at the PM have been identified.

In this paper we describe the specific participation of CoQ in trans-PM electron transport and the purification to homogeneity of a CoQ reductase from isolated pig-liver PMs. We also present evidence for the participation of the purified protein in trans electron transport through a mechanism involving CoQ.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

EXPERIMENTAL PROCEDURES

PM Preparation. Crude membrane fractions were obtained from pig-liver homogenates. PM was then isolated by the two-phase partition method and purity was checked by marker enzyme analysis as described (5). Membranes were resuspended in 50 mM Tris·HCl, pH 7.6/10% (vol/vol) glycerol/1 mM phenylmethylsulfonyl fluoride (PMSF)/1 mM EDTA/0.1 mM dithiothreitol (DTT) and stored either under liquid nitrogen or at −86°C.

Extraction and Restoration of CoQ. CoQ_{10} was extracted with heptane from lyophilized pig-liver PMs as described by Norling *et al.* (6). CoQ_{10} in heptane was added to both extracted and unextracted membranes and the solvent was evaporated to allow incorporation of the quinone into the dessicated membranes. Membranes were then taken up in 50 mM Tris·HCl (pH 7.6) for the assays of dehydrogenase activities.

Oxidoreductase Activities. NADH-CoQ reductase was assayed at 37°C by spectrophotometrically monitoring CoQ reduction at 410 nm in 0.2 mM NADH/0.2 mM CoQ_0/80–100 μg of protein with membranes or 5–50 μl of column eluates/50 mM Tris·HCl, pH 7.6. An extinction coefficient of 0.7 $mM^{-1}\cdot cm^{-1}$ was used in calculations of specific activities. NADH-FeCN reductase was assayed as described (3).

NADH-ascorbate free radical (AFR) reductase was assayed by measuring NADH oxidation at 340 nm upon addition of 66×10^{-3} units of ascorbate oxidase to a reaction mixture containing 0.4 mM ascorbate. An extinction coefficient of 6.22 $mM^{-1}\cdot cm^{-1}$ was used.

NADH-cytochrome *c* oxidoreductase was assayed by measuring the increase in absorbance at 550 nm in 0.2 mM NADH/20 μM cytochrome *c*/1 mM KCN/80–100 μg of membrane protein/50 mM Tris·HCl, pH 7.6. An extinction coefficient of 29.5 $mM^{-1}\cdot cm^{-1}$ was used.

The involvement of glycoproteins was tested by adding wheat germ agglutinin at 10 μg/ml. The sum of the rates obtained without added electron acceptor and without sample enzyme was subtracted for the final calculation of specific activities.

Solubilization of Integral Membrane Proteins. Prior to detergent-solubilization, membranes were extracted with 0.5 M KCl/25 mM Tris·HCl, pH 7.6/1 mM EDTA/1 mM PMSF/0.1 mM DTT/10% glycerol to remove peripheral membrane proteins and adsorbed soluble proteins. The pellet obtained after centrifugation at 105,000 × *g* for 30 min at 4°C was

Abbreviations: AFR, ascorbate-free radical; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CoQ, coenzyme Q; DTT, dithiothreitol; PM, plasma membrane; PMSF, phenylmethylsulfonyl fluoride.

*Permanent address: Departamento de Ciencias Agroforestales, Universidad de Huelva, 21071-Huelva, Spain.
†Permanent address: Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.
‡To whom reprint requests should be addressed at: Departamento de Biología Celular, Facultad de Ciencias, Universidad de Córdoba, Avda. San Alberto Magno, s/n. 14004 Córdoba, Spain.

resuspended in buffer without KCl and glycerol at a protein concentration of ≈10 mg/ml.

For protein solubilization, a 10% stock solution of CHAPS {3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate} in water was added dropwise to a final concentration of 2–3%. Detergent-to-protein ratio was maintained at 3.5. After incubation for 1 h on ice with gentle stirring, solubilized proteins were separated by ultracentrifugation at 105,000 × *g* for 1 h at 4°C.

Size-Exclusion Chromatography. Solubilized proteins were first fractionated in a column (5 × 45 cm) of Sephacryl S-300 HR. The gel was preequilibrated with column buffer (25 mM Tris·HCl, pH 7.6/1 mM EDTA/1 mM PMSF/0.1 mM DTT/0.6% CHAPS) and, after application of the sample (40 ml), 15-ml fractions were collected. The flow rate was maintained at 50 ml/h. Calibration was made with thyroglobulin (669 kDa), apoferritin (443 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), and cytochrome *c* (12.4 kDa). Void and total volumes were calculated with dextran blue and FMN, respectively.

Ion-Exchange Chromatography. Fractions with peak activity that eluted in the included volume were directly applied to a 1.6 × 15 column of DEAE-Sepharose CL-6B preequilibrated with column buffer. After washing with 10 column volumes, elution was carried out with a 0–0.8 M KCl gradient (240 ml). Fractions of 5 ml were collected at a flow rate of 10 ml/h.

Affinity Chromatography. Peak fractions of dehydrogenase activity from the preceding chromatography step were first dialyzed against 10 mM potassium phosphate, pH 7.5/0.6% CHAPS/1 mM EDTA/0.5 mM PMSF/0.1 mM DTT (dialysis buffer) in small columns of Sephadex G-25 and then applied at 2 ml/h to a column (1 × 7 cm) of 5′-ADP-agarose preequilibrated with dialysis buffer. After washing with at least 10 column volumes of dialysis buffer, elution of bound activity was accomplished with 5 mM NADH. Fractions of 1 ml were collected.

Reconstitution. Detergent was removed from fractions of purified CoQ reductase by dialysis and then the enzyme (3 μg) was reconstituted into sonicated phospholipid liposomes (10 mg/ml) by the freeze/thaw/sonication procedure (7). In another series of experiments, whole PMs isolated from pig liver (0.1–0.2 mg) were supplemented with excess purified enzyme (0.7 μg) by freeze/thaw/sonication.

Polyacrylamide Gel Electrophoresis. Purification was monitored by SDS/PAGE (10% polyacrylamide gels) as described by Laemmli (8). Samples were precipitated with trichloroacetic acid, suspended in SDS/DTT loading buffer [60 mM Tris·HCl, pH 6.8/bromophenol blue (0.1 mg/ml)/10% (wt/vol) sucrose/2 mM EDTA/20 mM DTT/1.5% (wt/vol) SDS], and heated at 42°C for 10 min to avoid aggregation of membrane proteins. Gels were stained with Coomassie blue R-250.

Protein determinations were carried out by the dye-binding method of Bradford (9), as modified by Stoscheck (10) for membrane samples, with bovine γ-globulin as standard.

RESULTS

PM Fractions. Fractions isolated by the two-phase partition method were highly enriched in PMs as revealed by enzyme marker analysis. The specific activity of ouabain-sensitive Na^+,K^+-ATPase, a marker for PMs, was increased ≈50-fold relative to the total homogenate. Specific activities of marker enzymes for mitochondria, endoplasmic reticulum, and Golgi apparatus were significantly decreased in PM fractions relative to the total homogenate.

Role of the CoQ Status in Trans- and Cis-PM Electron Transport. NADH-AFR reductase was assayed as an activity representing trans-PM electron transport, whereas NADH-cytochrome *c* oxidoreductase was assayed to measure cis electron transport through the cytochrome b_5 reductase–cytochrome b_5 system on the cytoplasmic side of the PM. Upon extraction of CoQ from PMs, NADH-AFR reductase activity was significantly inhibited. Addition of CoQ_{10} in heptane to lyophilized and extracted PMs with subsequent evaporation of the solvent restored the activity. Addition of CoQ_{10} in ethanol to extracted membranes in assay buffer, followed by an incubation period of 3–5 min to allow for incorporation of the quinone, also restored NADH-AFR reductase activity. When unextracted PMs were supplemented with extra CoQ, the activity was further stimulated. Unlike NADH-AFR reduc-

Table 1. Requirements for CoQ in PM redox activities

Acceptor	Activity, nmol per min per mg			
	C	E	R	S
FeCN	712	453 (−36)	770 (+8)	897 (+26)
AFR	7.48	4.48 (−40)	8.08 (+8)	11.9 (+59)
Cyt *c*	59.3	52.5 (−11)	52.5 (−11)	56.9 (−4)

Membranes were lyophilized (control, C) and extracted with heptane for 6 h at 20°C in the dark (extracted, E). Heptane was then decanted and evaporated. CoQ_{10} was added back to the membrane in heptane (reconstituted, R), and the heptane was removed by evaporation. Addition of excess CoQ_{10} to unextracted membranes was also carried out (supplemented, S). Values in parentheses are differences in redox activities vs. control membranes (in percent). Negative values indicate inhibition, whereas positive values indicate activation. Standard deviations were <10% (n = 3). Cyt *c*, cytochrome *c*.

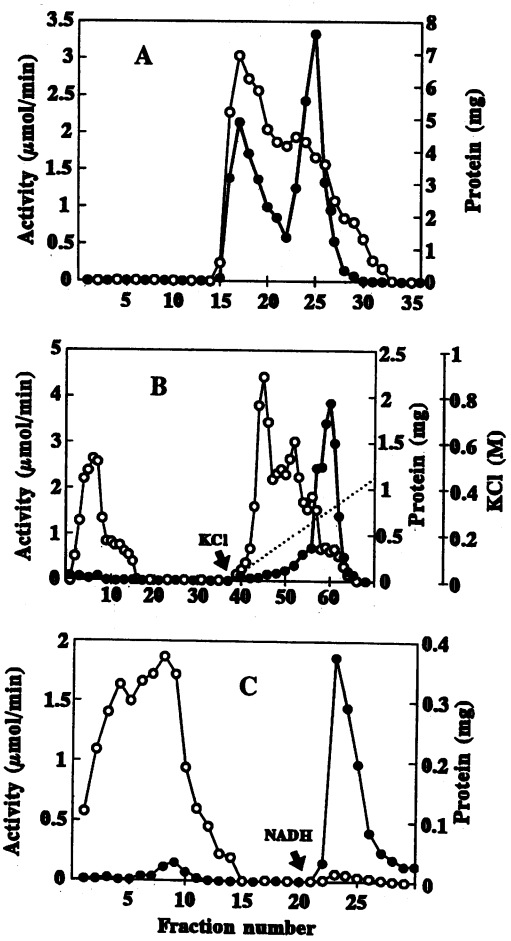

FIG. 1. Purification of the NADH-CoQ oxidoreductase by column chromatography. (*A*) Size-exclusion chromatography. (*B*) Ion-exchange chromatography. Bound proteins were eluted with a 0–0.8 M KCl gradient as indicated. (*C*) Affinity chromatography. Elution was carried out with 5 mM NADH. ●, NADH-CoQ_0 oxidoreductase activity; ○, protein profile; dotted line, KCl concentration.

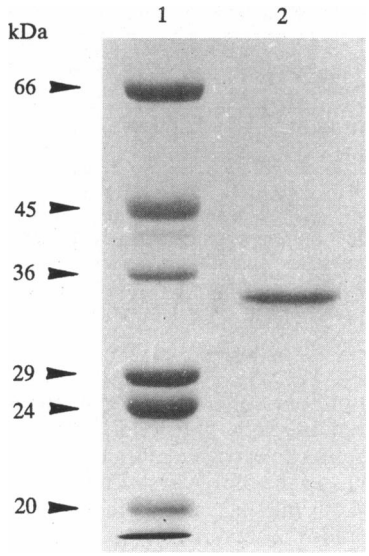

FIG. 2. SDS/PAGE. Lanes: 1, molecular mass standards; 2, 2 μg of purified CoQ reductase. After electrophoresis, the gel was stained with Coomassie blue. The molecular mass of the purified reductase is 34 kDa.

tase, NADH-cytochrome *c* oxidoreductase did not respond to the CoQ status of the PM, being insensitive to both extraction and addition of CoQ (Table 1).

NADH-AFR reductase activity was sensitive to quinone analogs. Both capsiacin at 150 μM and dicumarol at 200 μM inhibited the activity ≈40% and the inhibition was reversed by CoQ.

Solubilization and Purification of CoQ Reductase. After salt extraction, most CoQ reductase activity was recovered in the extracted membranes and solubilized with the zwitterionic detergent CHAPS. However, NADH-AFR oxidoreductase activity could not be recovered in a solubilized form, even in the presence of sonicated phospholipids.

Solubilized samples were first fractionated by molecular size with a column of Sephacryl S-300 HR (Fig. 1*A*). NADH-CoQ and -FeCN oxidoreductases showed parallel elution patterns, with two peaks of reductase activity. One was positioned at the void volume and ran in parallel with the protein elution pattern, thus, likely corresponding to high molecular weight protein aggregates and partially solubilized membranes. A second peak was eluted in the included volume, corresponding to a molecular mass of ≈70 kDa.

Fractions from the second peak (70 kDa) were loaded directly onto a column of DEAE-Sepharose CL-6B resulting in the binding of redox activities to the anion exchanger. After extensive washing with CHAPS-containing buffer, bound activity was eluted with a linear gradient of KCl (0–0.8 M). FeCN and CoQ reductases were coeluted at ≈0.3 M KCl (Fig. 1*B*).

Active fractions from ion-exchange chromatography were dialyzed to remove salt and applied to a column of 5′-ADP-agarose. After washing of the column, bound activity was eluted with 5 mM NADH (Fig. 1*C*). As in the preceding chromatography steps, NADH-quinone and -FeCN oxidoreductases were coeluted. Specific activities and recoveries of CoQ reductase during the purification procedure are summarized in Table 2.

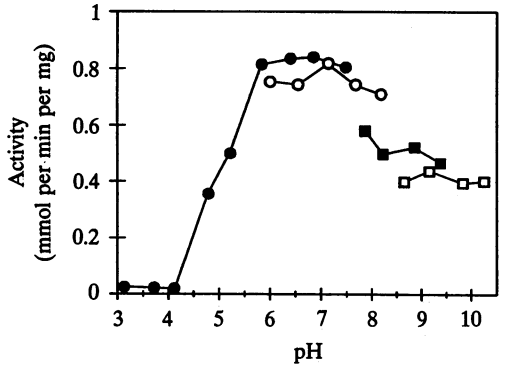

FIG. 3. pH curve of the purified reductase. Activity measured was NADH-FeCN oxidoreductase. Assays were carried out with 1 μg of purified reductase. ●, 40 mM citrate/phosphate buffer; ○, 40 mM phosphate buffer; ■, 40 mM Tris·HCl; □, 40 mM glycine·NaOH buffer.

Analysis by SDS/PAGE (Fig. 2) showed that affinity-purified fractions contained a single polypeptide with an apparent molecular mass of ≈34 kDa (p34).

Characterization of the CoQ Reductase. p34 was highly specific for NADH as electron donor. No significant activity was obtained with NADPH. Maximal rate of electron transfer was achieved with FeCN (0.7 mmol per mg per min). CoQ_0 was reduced at a rate of ≈95 μmol per mg per min. Other quinones such as juglone, duroquinone, and menadione were also reduced by p34, although at lower rates. The enzyme did not reduce AFR.

Reductase activity of p34 showed a broad optimal pH range between ≈6 and 7.5. Enzymatic activity was more sensitive to acidic than alkaline pHs. A sharp decline in enzymatic activity at pH <5.8 was observed (Fig. 3). K_m values for NADH and CoQ_0 were calculated from reciprocal plots and estimated to be ≈8 μM for NADH and 625 μM for CoQ_0. Biphasic saturation curves were found for both NADH and CoQ_0.

Spectrum analysis of the purified CoQ reductase suggested that the enzyme contained flavin although absorbance at 380 nm was higher than in other flavoenzymes (Fig. 4). The spectrum of the oxidized p34 had absorption maxima at 386 and 490 nm with a shoulder at ≈460 nm. Incubation in 20 μM NADH resulted in a rapid disappearance of these peaks. Absorption maxima were again detected after reoxidation with 50 μM ammonium persulfate.

Purified p34 was sensitive to quinone analogs. Capsiacin at 150 μM and dicumarol at 200 μM inhibited the NADH-FeCN reductase activity ≈30%. Inhibition with 0.5 mM chloroquine was ≈20%. The thiol reagent *p*-hydroxymercuribenzoate com-

Table 2. Purification of NADH-CoQ oxidoreductase from pig-liver PMs

Fraction	Protein, mg	Total activity, μmol/min	Specific activity, μmol per min per mg	Yield, %	Fold enrichment
PM	250	37.5	0.15	100	1
Salt-extracted	140	36.5	0.26	97.3	1.73
Solubilizate	72	24.5	0.38	65.3	2.53
Gel filtration	18	8.8	0.88	23.5	5.86
Ion exchange	1.25	8.4	11.9	22.4	79.3
Affinity	0.066	5.6	95.4	15	636

Assays for NADH-CoQ_0 oxidoreductase were carried out with samples of starting PMs, salt-extracted PMs (in the presence of 0.5% CHAPS for both), CHAPS solubilizates, and peak fractions obtained in successive steps of the purification procedure as indicated.

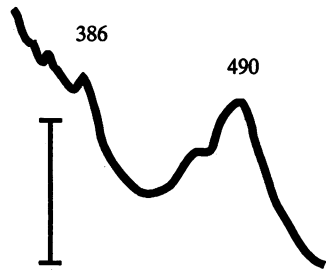

FIG. 4. Spectrum of oxidized form of purified PM CoQ reductase. Spectrum of reductase (0.2 mg/ml) was recorded in 10 mM potassium phosphate, pH 7.5/1 mM PMSF/1 mM EDTA/0.6% CHAPS. Positions of absorption peaks (in nm) are indicated. Bar indicates 0.003 OD unit.

pletely inhibited the activity at 0.1 mM but little inhibition was observed at 1 μM, a concentration that completely inhibits NADH-cytochrome b_5 reductase. Incubation of purified p34 with FAD, FMN, or riboflavin did not increase redox activity. No inhibition was obtained with the lectin wheat germ agglutinin at concentrations up to 10 μg/ml.

Reconstitution. CoQ_{10} was not reduced by purified p34 in assay buffer, but when 10 μM CoQ_{10} was added in ethanol to phospholipid liposomes containing p34, a rapid reduction of the quinone (measured at 410 nm) was observed after addition of 50 μM NADH (Fig. 5). No reduction signals were detected in control experiments with protein-free liposomes or after addition of ethanol alone.

Role of p34 in Trans-Membrane Electron Transport. To demonstrate the role of the CoQ reductase p34 in trans-PM electron transport, we added an excess of purified p34 to isolated PMs and assayed NADH-AFR and -cytochrome *c* oxidoreductase activities.

When excess p34 was added to PMs, NADH-AFR reductase was activated. This effect was related to both the CoQ content and the activity of added p34 since (*i*) it was best observed with CoQ_{10}-supplemented PMs and (*ii*) boiled p34 did not activate NADH-AFR reductase (Table 3). NADH-AFR reductase was not activated after addition of excess p34 to CoQ-depleted PMs. NADH-cytochrome *c* oxidoreductase was insensitive to an increased amount of p34, thus supporting the idea that activation of NADH-AFR reductase was not mediated by cytochrome b_5. Nonstimulated, CoQ_{10}-stimulated, and p34-stimulated NADH-AFR reductase activities were inhibited by the lectin wheat germ agglutinin at 10 μg/ml. This suggests the participation of an additional glycoprotein component in the overall reaction between NADH and AFR.

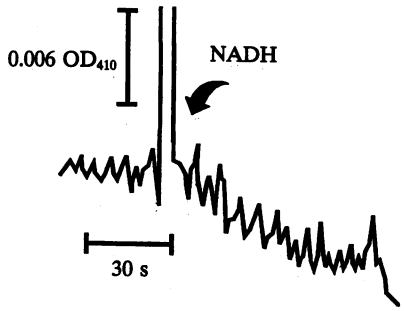

FIG. 5. Reconstitution of CoQ_{10}-reductase activity. Purified CoQ reductase (3 μg) was reconstituted by freeze/thaw/sonication into liposomes preformed by clarification of a solution of purified phospholipids (10 mg/ml) under nitrogen. CoQ_{10} was added in ethanol to 10 μM. Final concentration of ethanol was <1%. Liposomes were preincubated for 3 min to allow for incorporation of the quinone and then the reaction was initiated with NADH.

Table 3. Activation of trans-PM electron transport by p34

Activity	Activation, %	
	p34	Boiled p34
NADH-AFR	+60	−13
NADH-Cyt *c*	−9	−2

p34 was dialyzed to remove detergent, and then the protein (0.7 μg) was incubated with CoQ_{10}-supplemented membranes to allow incorporation into the PMs. Values represent differences in redox activities vs. CoQ_{10}-supplemented membranes without addition of p34. Positive values are the mean activation, whereas negative values are the mean inhibition. Standard deviations were <15% (n = 3). Cyt *c*, cytochrome *c*.

DISCUSSION

The ubiquitous presence of CoQ in animal membranes is now firmly established although its exact role in membranes other than the inner mitochondrial membrane is still controversial (1). In addition to a general role in storage or as an antioxidant molecule (2), CoQ may act as an electron carrier in some PM-associated redox reactions (3).

In this work we have demonstrated that the participation of CoQ as electron carrier is specific to trans-PM electron transport. NADH-AFR reductase represents a trans-membrane flux of electrons from cytoplasmic NADH to extracellular AFR (11). On the other hand, NADH-cytochrome *c* reductase represents electron transport between cytochrome b_5 reductase and cytochrome b_5 on the cytoplasmic side of the PM (12). Extraction of CoQ from PM inhibited trans but not cis electron transport. Addition of CoQ restored trans activity. The specific role of CoQ in trans-PM electron transport is further supported by inhibition of NADH-AFR reductase by quinone analogs and can be the basis for the stimulation of cell growth by CoQ in serum-free medium (3, 13). Growth stimulation by other external impermeable oxidants that activate trans-PM redox activity such as FeCN or AFR has been also reported (14, 15).

Molecular characterization of trans-PM redox systems has developed recently. Systems involved in the reduction and uptake of extracellular iron by the yeast *Saccharomyces cerevisiae* begin to be understood (16), and two genes encoding for PM ferric reductases (*FRE1* and *FRE2*) have been cloned and sequenced (17, 18). In plant systems, two redox proteins have been recognized at the PM and purified to homogeneity (19). However, the knowledge of those constituents involved in redox reactions at the animal PM is still incomplete.

Here, we have shown that a flavoprotein of 34 kDa is responsible for PM-associated CoQ reduction. This enzyme is important because it is a unique, low molecular weight, nonheteropolymeric enzyme in the PM for reduction of CoQ, providing a readily available source of antioxidant regeneration in the PM. It can further be the basis for trans electron transport, which has been described in the PM and requires CoQ (ref. 3 and this work).

The enzyme is an integral membrane protein since it is resistant to salt extraction. The apparent molecular mass estimated by gel filtration under nondenaturing conditions is ≈70 kDa, so p34 may form a dimer upon solubilization with CHAPS. p34 is not able to reduce CoQ_{10} in solution, probably due to the very low solubility of the acceptor in aqueous solutions. However, in reconstituted systems p34 catalyzed a very rapid NADH-dependent reduction of CoQ_{10}.

Kitajima *et al.* (20) purified a 36-kDa NADH-FeCN oxidoreductase from erythrocyte PMs and characterized it as a cytochrome b_5 reductase that is probably involved in keeping hemoglobin in its reduced state. To date, it has not been reported that cytochrome b_5 reductase displays CoQ-reductase activity. The purified CoQ reductase differs from the cytochrome b_5 reductase in several aspects. pH curves of NADH-FeCN reductase activity assayed with the two purified

proteins are very different; cytochrome b_5 reductase has an absorption maximum at 460 nm, whereas that of p34 is at 490 nm, indicating that the microenvironment of the flavin group is different in the two proteins. Also, the 34-kDa CoQ reductase exhibits a 100-fold increased resistance to the thiol reagent *p*-hydroxymercuribenzoate. NADH-AFR reductase of rat liver PMs also shows greater resistance to several thiol reagents that differentiate it from the cytochrome b_5 reductase (21). Incubation of purified p34 with FAD, FMN, or riboflavin did not increase redox activity, so if the cofactor is lost during purification, it cannot be easily replaced.

Although the enzyme lacked AFR reductase activity, supplementing PMs with an excess of p34 resulted in an activation of the NADH-AFR reductase. NADH-cytochrome *c* oxidoreductase was not activated, indicating that most AFR was not reduced by the cytochrome b_5 reductase–cytochrome b_5 system in liver PMs but by a more complex sequence of enzymes (22). This or a similar enzyme might be the basis for maintaining CoQ in a reduced state in other endomembranes such as Golgi bodies, with consequent enhancement of antioxidant protection throughout the cell.

Note Added in Proof. A peptide obtained by digestion of the purified reductase has been sequenced by Gerald W. Becker, Melvin G. Johnson, and Warren C. Mackellar from Lilly Research Laboratories (Indianapolis). The sequence was identical to amino acids 24 to 39 in bovine microsomal NADH-cytochrome b_5 reductase.

This work was partially supported by the Spanish Comision Interministerial de Ciencia y Tecnologia Grant PB92-0714. F.N. and A.A. were granted by the Universidad de Córdoba. F.L.C. was supported by the Spanish Ministerio de Educación y Ciencia.

1. Kalin, A., Norling, B., Appelkvist, E. L. & Dallner, G. (1987) *Biochim. Biophys. Acta* **926,** 70–78.
2. Ernster, L. & Forsmark-Andrée, P. (1993) *Clin. Invest.* **71,** S60–S65.
3. Sun, I. L., Sun, E. E., Crane, F. L., Morré, D. J., Lindgren, A. & Löw, H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11126–11130.
4. Kishi, T., Fukui, K., Fukmaga, Y., Takahashi, T., Okamoto, T. & Yamagami, T. (1991) in *Biomedical and Clinical Aspects of Coenzyme Q*, eds. Folkers, K., Littarru, G. P. & Yamagami, T. (Elsevier, Amsterdam), pp. 104–114.
5. Navas, P., Nowack, D. D. & Morré, D. J. (1989) *Cancer Res.* **49,** 2147–2156.
6. Norling, B., Glazek, E., Nelson, B. D. & Ernster, L. (1974) *Eur. J. Biochem.* **47,** 475–482.
7. Jones, O. T., Earnest, J. P. & McNamee, M. G. (1987) in *Biological Membranes: A Practical Approach*, eds. Findlay, J. B. C. & Evans, W. H. (IRL, Oxford), pp. 139–177.
8. Laemmli, U. K. (1970) *Nature (London)* **277,** 680–685.
9. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
10. Stoscheck, C. M. (1990) *Methods Enzymol.* **182,** 50–68.
11. Navas, P., Estévez, A., Burón, M. I., Villalba, J. M. & Crane, F. L. (1988) *Biochem. Biophys. Res. Commun.* **154,** 1029–1033.
12. Steck, T. L. & Kant, J. A. (1974) *Methods Enzymol.* **31,** 172–180.
13. Martinus, R. D., Linnane, A. W. & Nagley, P. (1993) *Biochem. Mol. Biol. Int.* **31,** 997–1005.
14. Sun, I. L., Crane, F. L., Grebing, C. & Löw, H. (1985) *Exp. Cell Res.* **156,** 528–536.
15. Alcaín, F. J., Burón, M. I., Rodríguez-Aguilera, J. C., Villalba, J. M. & Navas, P. (1990) *Cancer Res.* **50,** 5887–5891.
16. Dancis, A., Yuan, D. S., Haile, D., Askwith, C., Eide, D., Moehle, C., Kaplan, J. & Klausner, R. D. (1994) *Cell* **76,** 393–402.
17. Dancis, A., Roman, D. G., Anderson, G. J., Hinnebusch, A. G. & Klausner, R. D. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3869–3873.
18. Georgatsou, E. & Alexandraki, D. (1994) *Mol. Cell. Biol.* **14,** 3065–3073.
19. Serrano, A., Córdoba, F., González-Reyes, J. A., Navas, P. & Villalba, J. M. (1994) *Plant Physiol.* **106,** 87–96.
20. Kitajima, S., Yasukochi, Y. & Minakami, S. (1981) *Arch. Biochem. Biophys.* **210,** 330–339.
21. Villalba, J. M., Canalejo, A., Burón, M. I., Córdoba, F. & Navas, P. (1993) *Biochem. Biophys. Res. Commun.* **192,** 707–713.
22. Villalba, J. M., Canalejo, A., Rodríguez-Aguilera, J. C., Burón, M. I., Morré, D. J. & Navas, P. (1993) *J. Bioenerg. Biomembr.* **25,** 411–417.