Journal of Bioenergetics and Biomembranes, Vol. 29, No. 1, 1997

Original Article

Proton Pumping of Mitochondrial Complex I: Differential Activation by Analogs of Ubiquinone

Leon Helfenbaum,[1,3] Anna Ngo,[1] Anna Ghelli,[2] Anthony W. Linnane,[1] and Mauro Degli Esposti[1]

Received January 7, 1996; accepted July 1, 1996

As part of the ongoing studies aimed at elucidating the mechanism of the energy conserving function of mitochondrial complex I, NADH: ubiquinone (Q) reductase, we have investigated how short-chain Q analogs activate the proton pumping function of this complex. Using a pH-sensitive fluorescent dye we have monitored both the extent and initial velocity of proton pumping of complex I in submitochondrial particles. The results are consistent with two sites of interaction of Q analogs with complex I, each having different proton pumping capacity. One is the physiological site which leads to a rapid proton pumping and a stoichiometric consumption of NADH associated with the reduction of the most hydrophobic Q analogs. Of these, heptyl-Q appears to be the most efficient substrate in the assay of proton pumping. Q analogs with a short-chain of less than six carbons interact with a second site which drives a slow proton pumping activity associated with NADH oxidation that is overstoichiometric to the reduced quinone acceptor. This activity is also nonphysiological, since hydrophilic Q analogs show little or no respiratory control ratio of their NADH:Q reductase activity, contrary to hydrophobic Q analogs.

KEY WORDS: NADH: ubiquinone reductase; ubiquinone; proton pumping; mitochondria.

INTRODUCTION

Mitochondrial complex I, NADH: ubiquinone (Q) reductase, is associated with the first coupling site of oxidative phosphorylation (Walker, 1992; Ohnishi, 1993). Electron transport to ubiquinone is coupled to proton pumping with a stoichiometry that was initially reported to be one proton per electron (Mitchell, 1966; Lawford and Garland, 1972; Ragan and Hinkle, 1975), and later revised to two protons per electron (Di Virgilio and Azzone, 1982; Walker, 1992; Degli Esposti and Ghelli, 1994). Several models have been advanced to explain how protons are translocated by complex I (for a review, see Walker, 1992). Most of these models have assumed that the FMN and iron-sulfur cofactors of complex I are directly involved in proton pumping (Weiss *et al.*, 1991; Walker, 1992; Vinogradov, 1993). This assumption is in conflict with current knowledge that all the cofactor-binding subunits of complex I are proteins which do not trasverse the membrane, and are therefore unlikely to form a proton pump (Walker, 1992; Finel, 1993). On the other hand, mitochondrially-encoded subunits of complex I are integral transmembrane proteins which are presumed to form the Q reacting site and participate in proton pumping (Walker, 1992; Finel, 1993; Degli Esposti and Ghelli, 1994).

To investigate the critical role of Q reduction in the energy-conserving function of complex I, we have previously analyzed the specificity of a variety of short-chain analogs of ubiquinone for the reductase

[1] Centre for Molecular Biology and Medicine, Monash University, Clayton, 3168 Victoria, Australia.
[2] Department of Biology, University of Bologna, Bologna, Italy.
[3] Current address: Department of Biochemistry and Molecular Biology, University of Melbourne, Parkville, 3052 Victoria, Australia.

0145-479X/97/0200-0071$12.50/0

activity of the complex (Degli Esposti *et al.*, 1996). Q analog substrates were found to react at two sites, only one of which is presumed to overlap the site of action of endogenous Q. The other site preferentially reacts with hydrophilic Q analogs (quinones with a short chain of less than six carbons) and is less efficient in generating membrane potential. In this study we extend the investigation of the specificity of ubiquinone analogs by characterizing their activation of the proton pumping function of complex I. The present data support the concept of two distinct modes of interaction of exogenous quinones with the energy-conserving function of NADH:Q reductase.

MATERIALS AND METHODS

The 6-substituted analogs of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q-*0*) were synthesized and characterized as described previousy (Degli Esposti *et al.*, 1996). The isoprenoid Q analogs, Q-*1* and Q-*2*, were generous gifts of Eisai Co., Tokyo, Japan and the decyl-analog was purchased from Sigma Co., St. Louis, Missouri, USA. The undecyl-analog was the kind gift of Dr. E. Berry, University of California, Berkeley, California, USA. Quinones were stored in ethanolic solutions in the dark at −80°C. An average extinction coefficient of 14.5 $mM^{-1}cm^{-1}$ at 275–280 nm was utilized for determining the concentration of all Q analogs except for Q-*0*, for which an extinction coefficient of 13.8 $mM^{-1}cm^{-1}$ at 265 nm was used (Degli Esposti *et al.*, 1996). Methoxy-acrylate-stilbene was kindly given by Dr. P. Rich, Bodmin Research Institute, Cornwall, UK. Carboxin was a gift of Dr. J. Hargreaves, AFRC, Long Ashton, UK. The concentration of all inhibitors was determined spectrophotometrically in absolute ethanol using the reported extinction coefficients (Degli Esposti *et al.*, 1994b and references therein).

Submitochondrial particles from beef heart were prepared using essentially the procedure of Hansen and Smith (1964) for electron transport particles (ETP_H)[4] (Degli Esposti *et al.*, 1994b, 1996). The particles were usually washed by centrifugation with sucrose-Tris buffer containing 1% bovine serum albumin, and then stored at −70°C. The NADH:Q reductase activity of ETP_H was assayed as described recently (Degli Esposti *et al.*, 1994b, 1996). The electric membrane potential generated by NADH:Q reductase was measured optically with oxonol VI at 630–601 nm (Bashford and Smith, 1979; Degli Esposti *et al.*, 1996). The ETP_H were first diluted to 6–12 mg/ml and treated with 2 nmol of oligomycin per mg of protein to block proton leak through the ATP-synthase complex and by 1 nmol of antimycin plus 3 nmol of methoxy-acrylate-stilbene to block both Q sites in complex III (Degli Esposti *et al.*, 1993, 1996). The particles were then adjusted to a final protein concentration of 0.1–0.15 mg/mL in the assay medium, consisting of 0.125 M sucrose, 0.05 M Tricine-OH, 0.04 M KCl, and 2.5 mM $MgCl_2$, pH 8 (sucrose-Tricine buffer), containing 1 μM nigericin and 3 μM oxonol-VI. The transient optical changes associated with NADH oxidation were completely abolished by protonophore uncouplers (cf. Bashford and Smith, 1979).

The proton pumping activity of ETP_H was measured at 22–24°C by following the fluorescence quenching of the pH-sensitive dye, 9-amino-6-chloro-2-methoxyacridine (ACMA) at a concentration of 1.5–2 μM with particles treated as in the membrane potential assays (Ghelli, 1994; Degli Esposti and Ghelli, 1994). The particles were used at a final concentration of 0.4–0.5 mg/ml in the sucrose-Tricine buffer normally supplemented with 0.7 μM valinomycin; in most experiments, 20–25 μM carboxin was also added to the medium to inhibit complex II. After the addition of 0.1 mM NADH, proton pumping of complex I was initiated by the addition of Q analogs at different concentrations (see Fig. 1). In some experiments, sequential additions of 2–20 μM of a given Q analog were performed. ACMA fluorescence was measured in a Perkin-Elmer LS50B luminescence spectrometer under constant stirring with 412 nm (bandwidth of 2.5 nm) as the excitation wavelength and 510 nm (bandwidth of 5 nm) as the emission wavelength. Some experiments were carried out in a Jasco fluorimeter under similar settings but with wider bandwidths (cf. Casadio, 1991). The quenching of the ACMA signal was calibrated by direct measurements of pH as described previously (Casadio, 1991). The percent quenching increased linearly with the ΔpH from 0 to 0.8 pH units, with a slope of ca. 5% quenching per 0.1 pH unit, and then had a nonlinear pattern similar to that reported in bacterial chromatophores

[4] ABBREVIATIONS: ACMA, 9-amino-6-chloro-2-methoxyacridine; Δ*p*, protomotive force; ETP_H, electron transfer submitochondrial particles; Δψ, electric membrane potential; Q_{3A}, propenyl-Q; Q_{3B}, propyl-Q; Q_{5C}, pentyl-Q; Q_{6C}, hexyl-Q; Q_{7C}, heptyl-Q; Q_{8C}, octyl-Q; Q_{9C}, nonyl-Q; Q_{10C}, decyl-Q (or DB); Q_{11C}, undecyl-Q (or UBQ); Q, ubiquinone or coenzyme Q-*10*; RCR, respiratory control ratio.

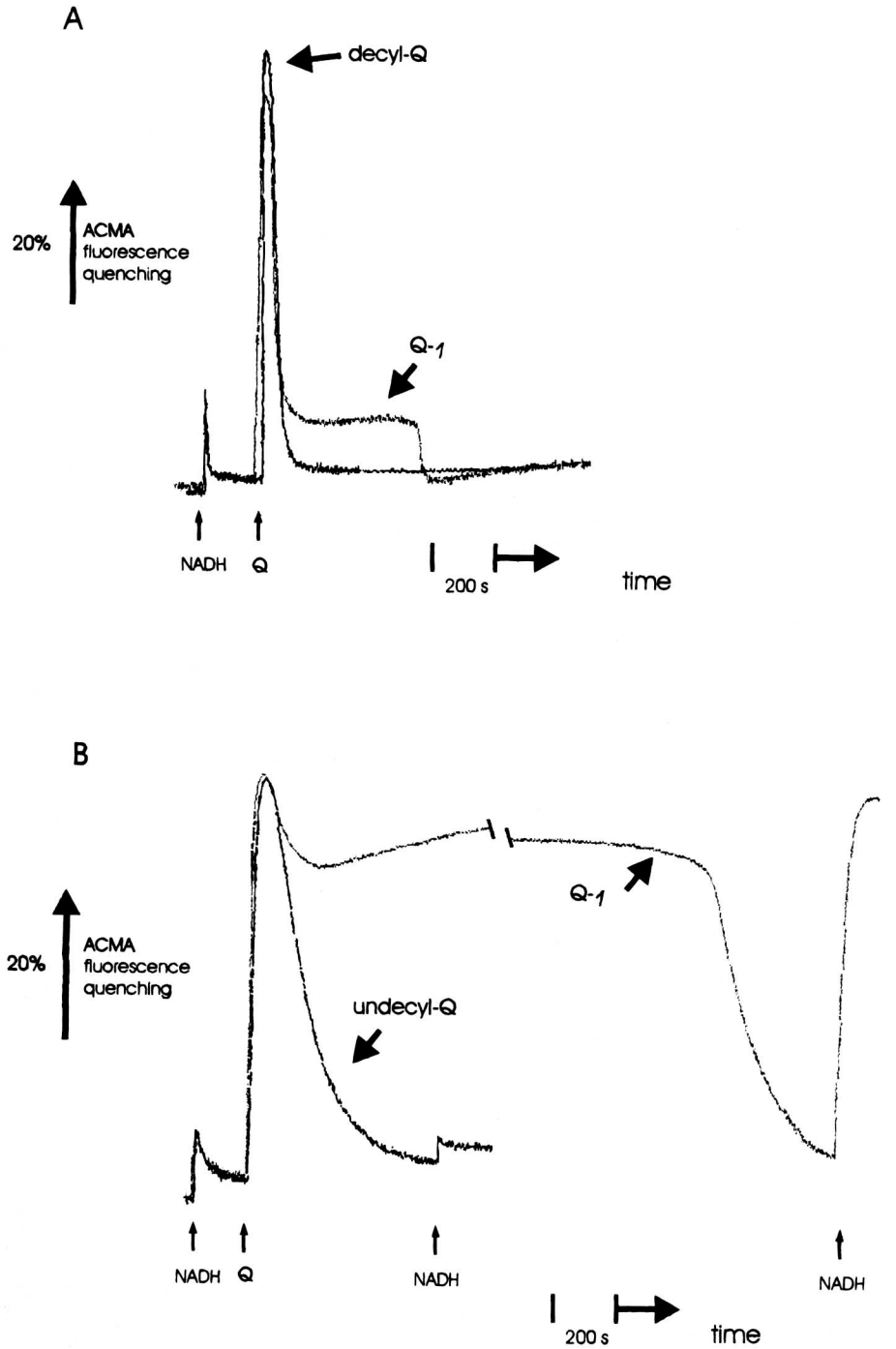

Fig. 1. Fluorescence changes of ACMA due to proton pumping of complex I. 0.4 mg/ml of tightly-coupled particles were incubated in the sucrose–Tricine buffer containing 20 μM carboxin and reduced by 100 μM NADH, followed by the addition of a hydrophobic Q analog (darker trace) or of Q-*1* (lighter trace) A, in the presence of 0.7 μM valinomycin, and with 40 μM Q analogs; B, in the absence of valinomycin, and with 20 μM of Q analogs. The results with decyl-Q and undecyl-Q are very similar with or without valinomycin, and show a monophasic return of ACMA fluorescence. The addition of Q-*1* produces instead a biphasic return in the fluorescence recovery of ACMA. This biphasic return is particularly pronounced in B, wherein a further addition of 100 μM NADH, after the fluorescence has returned to the initial level, reactivates the quenching of ACMA fluorescence. By contrast, further addition of NADH does not promote any nigericin-sensitive quenching of ACMA fluorescence when a hydrophobic Q analog such as undecyl-Q is used (B).

(cf. Casadio, 1991). Because of this lack of linearity, no transformation into ΔpH was normally undertaken for the large quenching signals generated by Q analogs. The quenching of ACMA fluorescence was completely abolished by submicromolar concentrations of nigericin.

Evaluation of the respiratory control ratio of the NADH:Q reductase activity was performed with 20 or

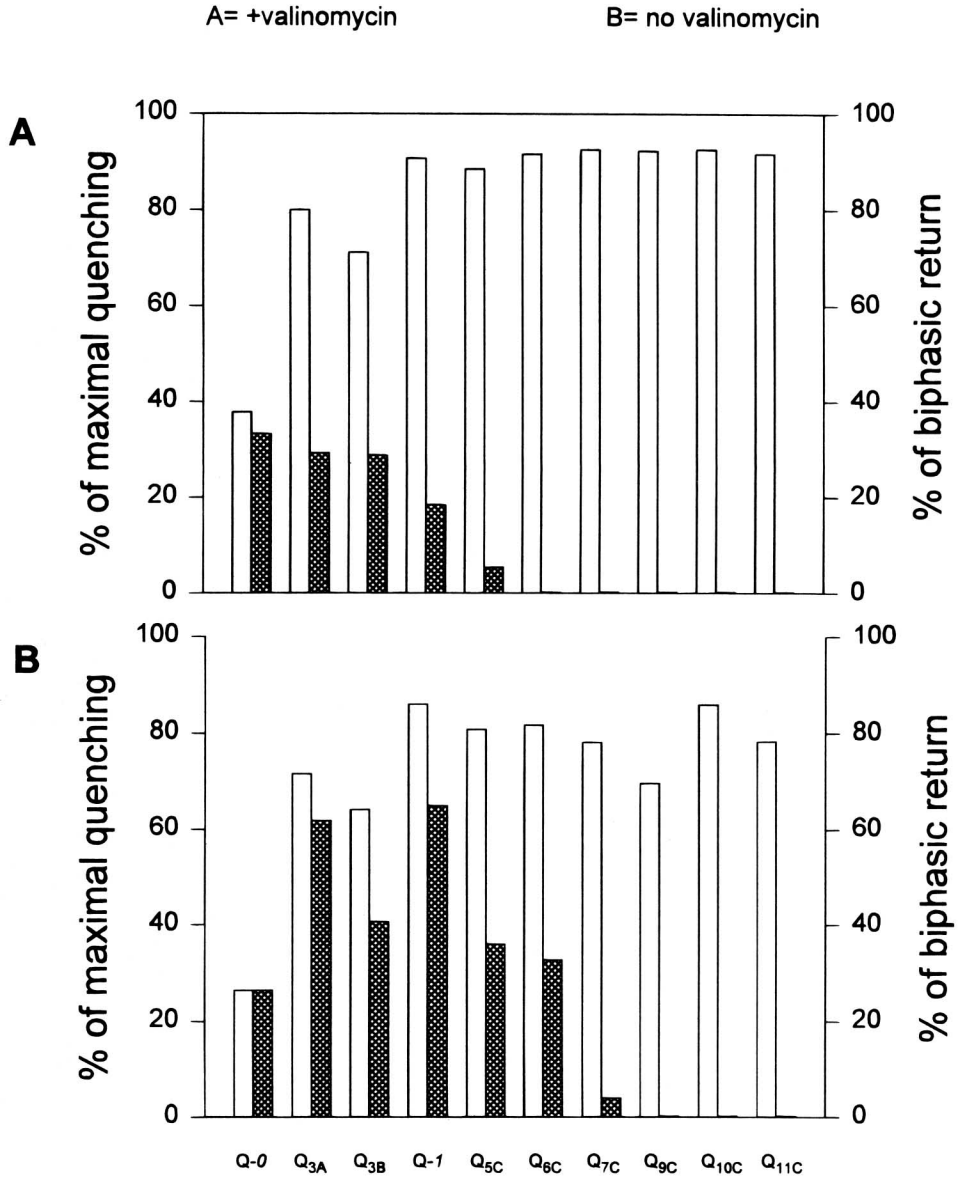

Fig. 2. Extents of ACMA fluorescence quenching with different Q analogs. A, in the presence of valinomycin. B, in the absence of valinomycin. The maximal extent of fluorescence quenching obtained by the addition of 20 μM of any Q analog is expressed as percent of the final fluorescence recovery (cf. Casadio, 1991). The experimental conditions are identical to those of Fig. 1B, with 20 μM carboxin in all cases. The hatched histograms represent the portion of ACMA signal that does not return monophasically to the initial fluorescence value as a reflection of the redox sink reaction consuming excess of NADH (cf. Fig. 1). Note that for levels beyond 75% quenching there is no accurate correspondence to ΔpH values due to the nonlinearity of the ACMA responses (Casadio, 1991). The Q analogs are shown in the increasing order of their hydrophobicity, (cf. Degli Esposti *et al.*, 1996).

40 μM Q analogs in sucrose-Tricine buffer containing ionophores at 0.7–1 μM.

RESULTS AND DISCUSSION

Measurements of the Proton Pumping of Complex I with ACMA and Effects of Hydrophilic Q Analogs

ACMA is a sensitive probe for monitoring the proton pumping activity of complex I in ETP_H preparations from beef heart (Degli Esposti and Ghelli, 1994). As illustrated in Fig. 1, saturating concentrations of both NADH and exogenous Q analogs promote an almost complete quenching of ACMA fluorescence due to proton pumping. Addition of the most hydrophobic Q analogs, i.e., those with a short chain of more than six carbons, produces a monophasic return to the initial level of fluorescence intensity. By contrast, more hydrophilic Q analogs such as Q-*1* activate a biphasic time course for the recovery of ACMA fluorescence, particularly in the absence of valinomycin (Fig. 1). In this case, the time course of ACMA quenching lasts for over half an hour while it remains fully sensitive to nigericin (results not shown). With the most hydrophilic quinones, the return of ACMA fluorescence actually shows a multiphasic behavior which sometimes results in oscillatory waves of fluorescence quenching and recovery (results not shown). When the fluorescence finally returns to the initial level, a further addition of NADH reactivates a cycle of nigericin-sensitive quenching (Fig. 1B). This suggests that the prolonged equilibrium of proton pumping maintained by the addition of a hydrophilic Q analog has completely exhausted the reducing substrate, even when it is well in excess of the Q acceptor, which evidently has not undergone complete reduction. However, hydrophobic Q analogs do not consume NADH in excess of their concentration, because further addition of NADH after complete recovery of ACMA fluorescence does not produce any quenching due to proton pumping—the small decrease in fluorescence is completely insensitive to nigericin, and thus is due to NADH interferences with the light emission of ACMA.

The biphasic (or multiphasic) return of ACMA fluorescence with the hydrophilic Q analogs is normally observed at a concentration higher than 4 μM when valinomycin is present in the assay medium, and its extent is inversely proportional to the hydrophobicity of the short-chain subsitutent (Fig. 2). This effect is also enhanced by the sequential addition of Q analogs in a single cuvette. In fact, the addition of a second aliquot of Q substrate after the complete reduction of the first aliquot results in the establishment of a biphasic return at lower concentrations than observed by individual Q additions in separate cuvettes (results not shown). The biphasic pattern of the return of ACMA fluorescence is not significant with Q analogs having an alkyl short chain of six or more carbons (especially in the presence of valinomycin, Fig. 2A).

With spectrophotometric measurements we have confirmed that the prolonged reaction of proton pumping promoted by hydrophilic Q analogs such as Q-*1* parallels a slow oxidation of NADH proceeding beyond the expected level of equilibrium of the redox reaction (i.e., overstoichiometrically). On the other hand, hydrophobic Q analogs such as decyl-Q essentially reach the expected stoichiometric equilibrium with the oxidation of NADH (data not shown). Consequently, Q-*1* and other hydrophilic Q analogs appear to interact with complex I as electron sinks in the NADH:Q reductase reaction, which is in accordance with our previous study on the Q analog specificity of the generation of membrane potential (Degli Esposti *et al.*, 1996). In general, the electron sink capacity derives from the fact that quinones are redox active compounds which can nonspecifically oxidize redox cofactors in complex I. Our data show that the redox sink capacity of hydrophilic Q analogs activates proton pumping, albeit more slowly and with a different behavior than that of hydrophobic Q analogs such as decyl-Q (Fig. 1).

The redox sink capacity could derive also from a preferred interaction of hydrophilic Q analogs with the site where the ubiquinol product is normally released by complex I—the site where ubiquinone enters the complex is too hydrophobic to be efficiently penetrated by these analogs (Ghelli, 1994; Degli Esposti and Ghelli, 1994). The interaction at the ubiquinol site could facilitate the incomplete reduction of hydrophilic Q analogs by complex I via semiquinones intermediates that are unstable and dismutate rapidly (Degli Esposti *et al.*, 1996). Dismutation produces both the fully-oxidized acceptor form and the fully-reduced quinol form of the Q analogs, which could react back with the complex and enhance the production of semiquinones. Hence, equilibrium of the redox reaction would not be reached in the presence of excess NADH reductant. Instead, a situation of oscillatory pseudo-equilibrium would

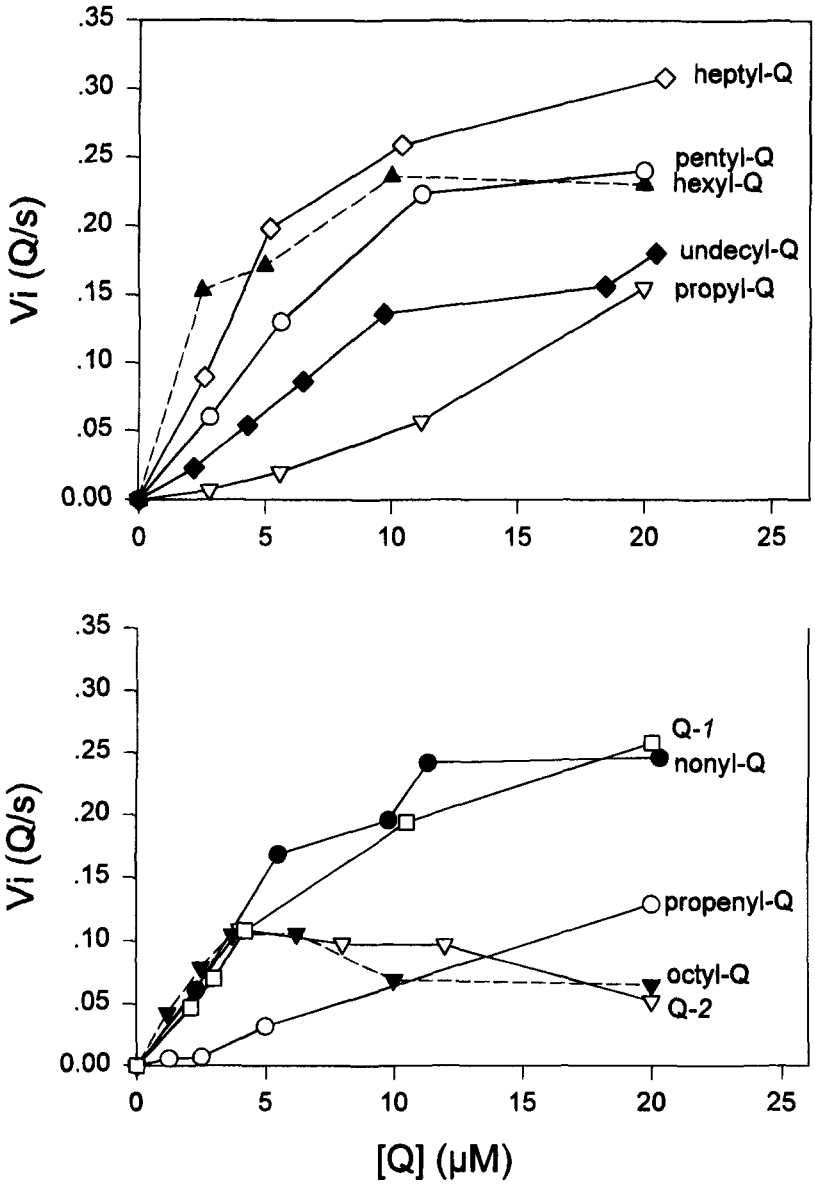

Fig. 3. Titrations of the initial velocity of proton pumping activated by different Q analogs. The initial velocities (V_i) are expressed in fractional quenching of ACMA fluorescence per second (Q/s). The experimental conditions were the same as those of Fig. 1 and 2, with valinomycin and carboxin present in all cases. The Q analogs were added at increasing concentrations within the same cuvette, after the fluorescence intensity returned to the equilibrium value. Titrations performed with additions in separate cuvettes generally gave slightly higher velocities of quenching (cf. Fig. 4), but the relative patterns of Q concentration dependence were superimposable on those shown in this figure. Note the substrate inhibition effect of both octyl-Q and Q-2 at concentrations higher than 5 μM. For other hydrophobic analogs similar effects were seen only at concentrations higher than 40 μM.

occur until NADH is completely oxidized via the continuous regeneration of exogenous Q acceptor due to dismutation. The pseudo-equilibrium of the redox reaction could maintain partially reduced some components of the complex which are involved in proton pumping, such as the stabilized semiquinones

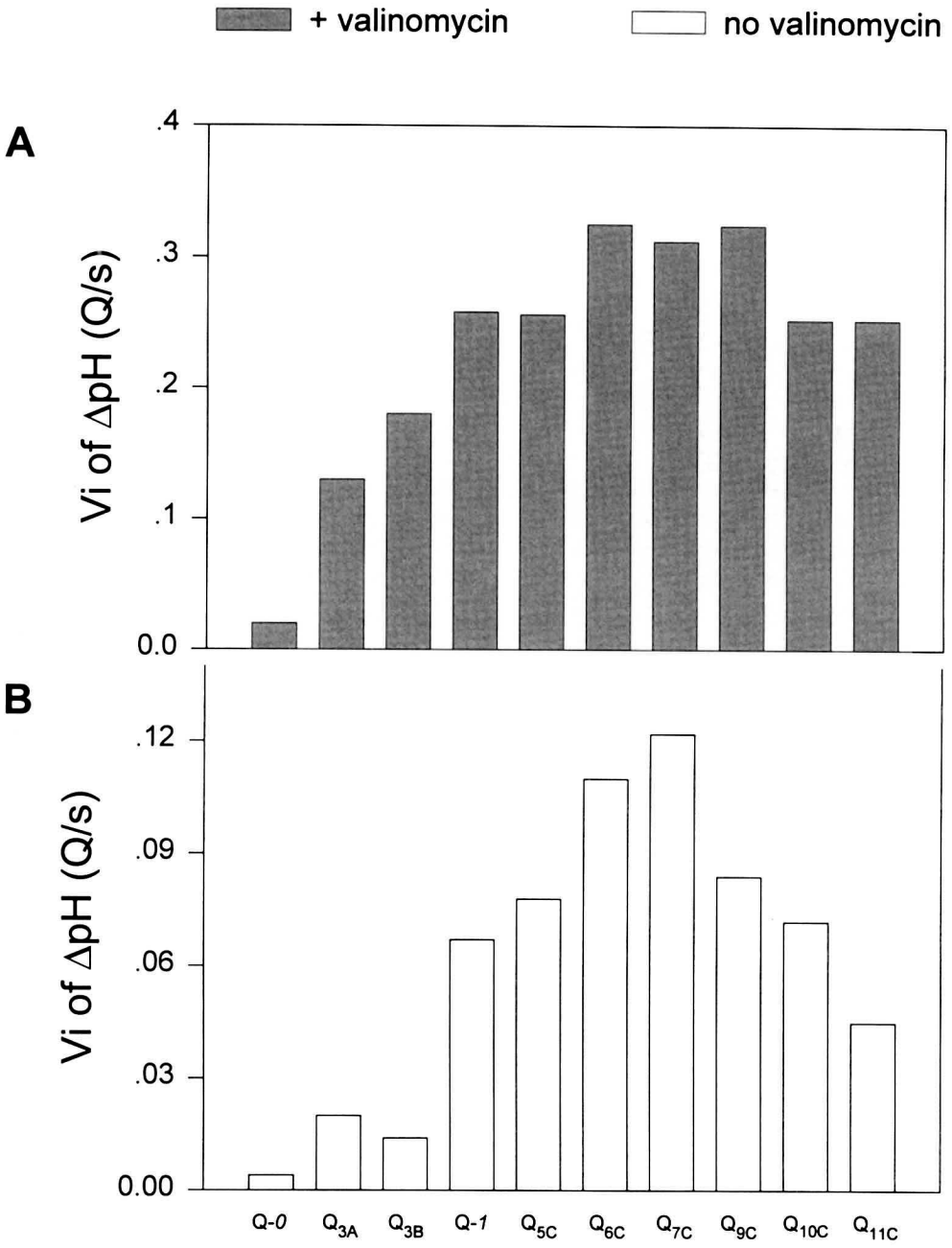

Fig. 4. Hydrophobicity profile of the proton pumping efficiency of Q analogs in complex I. A, in the presence of valinomycin. B, in the absence of valinomycin. The initial velocities of ΔpH are expressed as fractional quenching per second under experimental conditions identical to those of Fig 2. The Q analogs were all added at a final concentration of 20 μM after the signal induced by NADH reduction was completely reverted (cf. Fig. 1A). The Q analogs are shown in the increasing order of their hydrophobicity (cf. Degli Esposti *et al.*, 1996).

formed from endogenous ubiquinone in theoxidation of NADH (Suzuki and King, 1983; Kotlyar *et al.*, 1990; Degli Esposti and Ghelli, 1994). This explains the multiphasic or oscillatory behavior of ACMA fluorescence with hydrophilic quinones (cf. Fig. 1).

Q Specificity of the Proton Pumping Function of Complex I

Figure 3 shows the concentration dependence of the initial velocity of ACMA quenching with various

Q analogs added at increasing concentrations within the same cuvette. Generally, hydrophobic Q analogs with a short chain of more than five carbons saturate the rate of proton pumping at concentrations around 20 μM. Octyl-Q, however, reaches this saturation at approximately 5 μM, and shows substrate inhibition at higher concentrations; Q-2 is the only other analog that behaves similarly to octyl-Q (Fig. 3B). These Q analogs generate much faster rates when added directly at 20 μM without previous Q additions in the same cuvette, contrary to the majority of other Q analogs, for which additions in separate cuvettes generate rates of quenching that are slightly faster than those shown in Fig. 3 (cf. Figs. 3 and 4). Q analogs which are more hydrophilic than Q-*1* show no saturation in the velocity of ACMA fluorescence quenching when their concentration is increased up to 0.1 mM (Fig. 3 and results not shown), but this quenching seems to be due substantially to their electron sink capacity (cf. Fig. 1). Therefore, the measure of the initial velocity of ACMA quenching in this case may relate only partially to the physiological activity of proton pumping (see above and Degli Esposti *et al.*, 1996).

The dependence of the velocities of proton pumping upon the hydrophobicity of Q analogs has a bell-shaped profile (Fig. 4) which resembles that of the association constants of the NADH:Q reductase activity (Degli Esposti *et al.*, 1996). Similar bell-shaped profiles have been reported for 4-alkylacridones, complex I inhibitors that could act as Q antagonists (Oettmeier *et al.*, 1992). The maximum of these profiles corresponds to substituents with eight carbons for either Q analogs substrates (Degli Esposti *et al.*, 1996) or Q antagonist inhibitors (Oettmeier *et al.*, 1992). However, when assayed for proton pumping by complex I, octyl-Q is not only less efficient than either heptyl-Q or nonyl-Q (Fig. 3), but is also inhibitory of other Q analogs—this has also been described for Q-2 (Lenaz *et al.*, 1967; Estornell *et al.*, 1993). Consequently, we have excluded the use of octyl-Q and Q-*2* in most subsequent experiments.

Heptyl-Q shows the fastest rates of proton pumping among all the Q analogs tested in the absence of valinomycin (Fig. 4B)—i.e., when ACMA quenching is exclusively due to the ΔpH component of Δp (Casadio, 1991). Nonyl-Q is nearly as efficient as heptyl-Q and always activates faster rates of proton pumping than either decyl-Q or undecyl-Q (Figs. 3 and 4). Q analogs with a short chain of nine to eleven carbons were previously found to have the maximal efficiency in generating membrane potential of the NADH:Q reductase (Degli Esposti *et al.*, 1996). In parallel measurements we have confirmed here that heptyl-Q is consistently more efficient than decyl-Q in the ACMA assay of proton pumping (Fig. 4 and results not shown), while it generates slower rates than decyl-Q in the oxonol assay of electric membrane potential ($\Delta\psi$).

The relative difference in the energetic capacity of heptyl-Q and nonyl-Q as complex I substrates depends in part on the different physical sensitivity of the probe techniques used to monitor either the $\Delta\psi$ or the ΔpH component of Δp. In particular, we have usually observed that the rate and extent of oxonol changes are only marginally affected by micromolar concentration of nigericin (Ghelli, A., Benelli, B., and Degli Esposti, M., unpublished results). This indicates that the signals of the oxonol probe as measured here and previously (Degli Esposti *et al.*, 1996) essentially reflect the $\Delta\psi$ component (alone) of the Δp generated by the NADH:Q reductase activity.

The observation that heptyl-Q is less efficient than, e.g., decyl-Q in generating $\Delta\psi$ by complex I (Degli Esposti *et al.*, 1996) could derive also from a more effective inhibition of this function by the quinol product of heptyl-Q. Indeed, heptyl-Q attains saturation of the oxonol signal at a lower concentration than decyl-Q when the measurements are carried out with incremental additions within the same cuvette, which results in a progressive accumulation of the quinol product (results not shown). These considerations suggest the possibility that complex I has a different specificity for Q analogs in the reactions that activate proton pumping and in the reactions associated with charge separation across the membrane yielding $\Delta\psi$. Such a possibility seems to be confirmed by the results of an alternative experimental approach described below.

Respiratory Control Ratios of NADH:Q Reductase

ETP_H preparations normally show a significant stimulation of the rate of electron transport from NADH to hydrophobic Q analogs by protonic ionophores such as nigericin and gramicidin (Fig. 5). On the other hand, valinomycin increases the electron transport rate only slightly (Fig. 5A). It thus appears that the electron transport activity of complex I is controlled more by the ΔpH component than by the $\Delta\psi$ component of Δp. In contrast, the electron transport activity in *bc* and *bf* complexes is mainly controlled by the $\Delta\psi$ (cf. Hurt *et al.*, 1982). In coupled submitochondrial particles with inhibited complex III, the ionophore-induced stimulation of the NADH:Q reductase

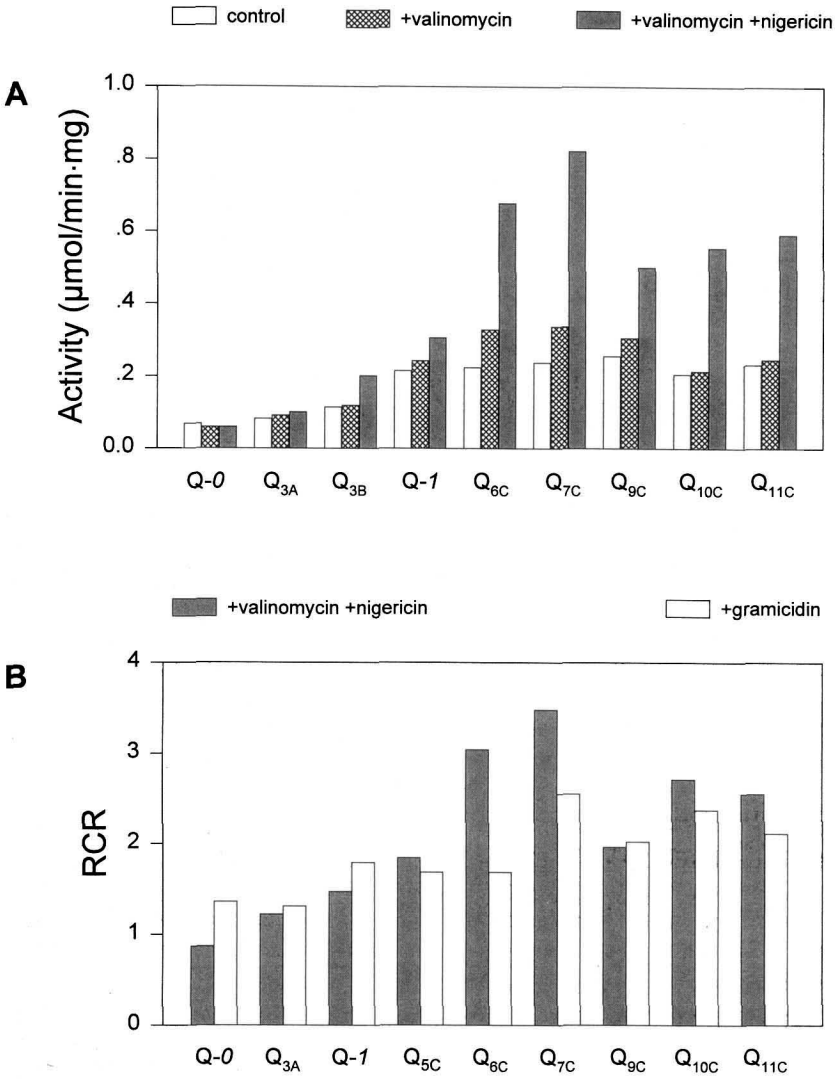

Fig. 5. Hydrophobicity profile of the respiratory control ratio of the NADH:Q reductase with different Q analogs. ETP_H were assayed for the NADH (100 µM):Q (40 µM) reductase at 0.04 mg/ml in the sucrose–Tricine buffer with no addition, or containing ionophores (0.6 µM valinomycin, 0.8 µM nigericin, and 1 µM gramicidin). A, absolute reductase rates in the absence (white histograms) and presence of either valinomycin alone (hatched histograms) or in combination with nigericin (gray histograms). B, values of the respiratory control ratios, which were obtained by dividing the rate in the presence of ionophores by the control rate in the absence of ionophores. The gray histograms represent the RCR values derived from the data obtained with a combination of valinomycin and nigericin in A, whereas the white histograms represents the RCR values from a separate experiment in which gramicidin alone was used to uncouple the particles and 20 µM carboxin was also present in the medium.

activity is phenomenologically equivalent to the respiratory control of the electron transport activity of isolated complex I inserted in lipid vesicles (Ragan and Hinkle, 1975). This respiratory control is measured as the ratio of the electron transport rate in the presence and absence of ionophores (RCR) and has different values with different Q analog substrates (Fig. 5). Various Q analogs, therefore, show a different energetic capacity in their interaction with complex I.

Complex I activity with hydrophilic Q analogs up to Q-*1* shows little respiratory control, since the RCR values are usually below 1.4 with any ionophore (Fig. 5B). A similar low RCR is consistently shown by Q-*2* as a substrate, probably due to its inhibitory

action on complex I (Lenaz *et al.*, 1967; Estornell *et al.*, 1993). The electron transport activity of complex I shows the highest RCR values with Q analogs having an alkyl short chain of six to eleven carbons (Fig. 5). With heptyl-Q the activity frequently shows the largest control ratios when nigericin (Fig. 5A and results not shown) or gramicidin (Fig. 5B) are used to uncouple the particles. The fact that hydrophilic Q analogs show little or no respiratory control of their NADH:Q reductase activity is consistent with previous measurements indicating that their energetic efficiency is clearly lower than that of the more hydrophobic Q analogs such as nonyl-Q (Degli Esposti *et al.*, 1996). The redox sink interaction with complex I which has been discussed earlier is the most likely cause of the energetic inefficiency of these hydrophilic Q analogs.

CONCLUSIONS

We suggested previously that complex I can reduce Q analogs at two sites, each having different structural specificities for the substituent at position 6 of the quinone ring (Degli Esposti *et al.*, 1996). One site preferentially reacts with hydrophilic Q analogs—i.e., those analogs having a side-chain of less than six carbons—and donates electrons to quinone acceptors primarily through the protein-bound semiquinone that is formed with the endogenous ubiquinone during the catalysis of complex I (Suzuki and King, 1983; Kotlyar *et al.*, 1990). The other site of Q interaction reacts specifically with hydrophobic Q analogs and presumably corresponds to the reaction site of the endogenous ubiquinone. This is the physiological site where ubiquinone coming from the membrane is fully reduced and proton pumping is efficiently activated in complex I (Degli Esposti *et al.*, 1996). The present results are consistent with this interpretation. In fact, hydrophilic quinones do not reach a normal redox equilibrium with complex I and sustain NADH oxidation also via dismutation of unstable semiquinones, thus activating proton pumping in a slow and multiphasic manner (Fig. 1). This proton pumping is partially nonphysiological, since the NADH:Q reductase activity of hydrophilic Q analogs show little or no respiratory control (Fig. 5).

The next step of our research is to investigate the relationship between the dual interaction of complex I with exogenous Q analogs and the action of the Q antagonist inhibitors of the complex.

REFERENCES

Bashford, C. L., and Smith, J. C. (1979). *Methods Enzymol.* **55**, 569–586.

Casadio, R. (1991). *Eur. Biophys. J.* **19**, 189–201.

Degli Esposti, M., and Ghelli, A. (1994). *Biochim. Biophys. Acta* **1187**, 116–120.

Degli Esposti, M., Ghelli, A., Crimi, M., Estornell, E., Fato, R., and Lenaz, G. (1993). *Biochem. Biophys. Res. Commun.* **190**, 1090–1096.

Degli Esposti, M., Crimi, M., and Ghelli, A. (1994a). *Biochem. Soc. Trans.* **22**, 209–213.

Degli Esposti, M., Ghelli, A., Ratta, M., Cortes, D., and Estornell, E. (1994b). *Biochem. J.* **301**, 161–167.

Degli Esposti, M., Ngo, A., McMullen, G., Ghelli, A., Sparla, F., Benelli, B., Ratta, M., and Linnane, A. W. (1996). *Biochem. J.* **313**, 327–334.

Di Virgilio, F., and Azzone, G. F. (1982). *J. Biol. Chem.* **257**, 4106–4113.

Estornell, E., Fato, R., Pallotti, F., and Lenaz, G. (1993). *FEBS Lett.* **332**, 127–131.

Finel, M. (1993). *J. Bioenerg. Biomembr.* **25**, 357–366.

Ghelli, A. (1994). Ph.D. thesis, University of Bologna.

Hansen, M., and Smith, A. L. (1964). *Biochim. Biophys. Acta* **81**, 214–222.

Hurt, E. C., Hauska, G., and Shahak, Y. (1982). *FEBS Lett.* **149**, 211–216.

Kotlyar, A. B., Sled, V. D., Burbaev, D. S., Moroz, I. A., and Vinogradov, A. D. (1990). *FEBS Lett.* **264**, 17–20.

Lawford, H. G., and Garland, P. B. (1972). *Biochem. J.* **130**, 1029–1044.

Lenaz, G., Daves, G. D., and Folkers, K. (1968). *Arch. Biochem. Biophys.* **123**, 539–550.

Mitchell, P. (1966). *Biol. Rev.* **41**, 445–502.

Oettmeier, W., Masson, K., and Soll, M. (1992). *Biochim. Biophys. Acta.* **1099**, 262–266.

Ohnishi, T. (1993). *J. Bioenerg. Biomembr.* **25** , 325–329.

Ragan, C. I., and Hinkle, P. C. (1975). *J. Biol. Chem.* **250**, 8472–8476.

Suzuki, H., and King, T. E. (1983). *J. Biol. Chem.* **258**, 352–358.

Vinogradov, A. D. (1993). *J. Bioenerg. Biomembr.* **25**, 367–375.

Walker, J. (1992). *Q. Rev. Biophys.* **25**, 253–324.

Weiss, H., Friedrich, T., Hofhaus, G., and Preis, D. (1991). *Eur. J. Biochem.* **197**, 563–576.