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# A Ubiquinone-binding Site Regulates the Mitochondrial Permeability Transition Pore*

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**We have investigated the regulation of the mitochondrial permeability transition pore (PTP) by ubiquinone analogues. We found that the $Ca^{2+}$-dependent PTP opening was inhibited by ubiquinone 0 and decylubiquinone, whereas all other tested quinones (ubiquinone 5, 1,4-benzoquinone, 2-methoxy-1,4-benzoquinone, 2,3-dimethoxy-1,4-benzoquinone, and 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone) were ineffective. Pore inhibition was observed irrespective of the method used to induce the permeability transition (addition of $P_i$ or atractylate, membrane depolarization, or dithiol cross-linking). Inhibition of PTP opening by decylubiquinone was comparable with that exerted by cyclosporin A, whereas ubiquinone 0 was more potent. Ubiquinone 5, which did not inhibit the PTP *per se*, specifically counteracted the inhibitory effect of ubiquinone 0 or decylubiquinone but not that of cyclosporin A. These findings define a ubiquinone-binding site directly involved in PTP regulation and indicate that different quinone structural features are required for binding and for stabilizing the pore in the closed conformation. At variance from all other quinones tested, decylubiquinone did not inhibit respiration. Our results define a new structural class of pore inhibitors and may open new perspectives for the pharmacological modulation of the PTP *in vivo*.**

The permeability transition is an *in vitro* increase of the inner mitochondrial membrane permeability to solutes with a molecular mass of up to ~1500 Da (1, 2). This phenomenon is now interpreted as being due to the opening of a proteinaceous, but yet unidentified, large conductance channel, the PTP.[1] PTP opening *in vitro* leads to the collapse of the proton-motive force, disruption of ionic homeostasis, mitochondrial swelling, and massive ATP hydrolysis by the $F_1F_0$-ATPase. This sequence of events has drawn considerable attention to the permeability transition as a potential player in the pathways to cell death (3–10). However, mitochondrial swelling might not be an essential feature of pore opening *in vivo*, and evidence is accumulating that the PTP may provide mitochondria with a fast $Ca^{2+}$-release channel (11–14). Indeed, conditions have been described under which the PTP is more selective and/or reversible (13, 15, 16), which may be of relevance to mitochondrial $Ca^{2+}$ signaling *in situ* (17, 18).

The PTP open-closed transitions are modulated by a variety of factors (matrix pH, transmembrane electrical potential, $Me^{2+}$ ions, $P_i$, the redox potential, and adenine nucleotides) at sites that can be discriminated, in part at least, through the effects of specific reagents (17). The permeability transition can also be induced or inhibited by a large variety of drugs (2). Among these, the most potent PTP inhibitor is the immunosuppressive peptide CsA (19–21), which became a diagnostic tool for the characterization of the PTP in isolated mitochondria and in living cells and organs. It is now clear, however, that pore inhibition by CsA becomes less efficient at increasing $Ca^{2+}$ loads (22) and that inhibition is only transient resulting in pore opening in longer time frame experiments (15). Furthermore, CsA does not selectively act on mitochondria and inhibits other cellular functions that depend on $Ca^{2+}$ and/or calcineurin (23).

We have recently shown that the PTP is regulated by electron flux through the respiratory chain Complex I independently of all known pore regulators and that $Ub_0$ inhibits the PTP opening induced by $Ca^{2+}$ overload (24). To characterize the mechanism of action of $Ub_0$ on the PTP, we have investigated the effect of commercially available quinones on the regulation of the permeability transition in isolated rat liver mitochondria. We tested quinones where carbon 6 of the benzoquinone ring was substituted with different side chains and where the radicals on carbons 2, 3, or 5 were partially or totally missing (Fig. 1). We found that the PTP opening was inhibited only by $Ub_0$ and decyl-Ub, irrespective of the method used to induce the permeability transition (addition of $P_i$ or atractylate, membrane depolarization, or dithiol cross-linking). Inhibition of the PTP opening by decyl-Ub was comparable with that exerted by CsA, whereas $Ub_0$ was more potent. $Ub_5$, which did not inhibit the PTP *per se*, specifically counteracted the inhibitory effect of $Ub_0$ or decyl-Ub but not that of CsA. These findings define a quinone-binding site directly involved in PTP regulation and indicate that different quinone structural features are required for binding and for stabilizing the pore in the closed conformation. Our results define a new structural class of pore inhibitors and may open new perspectives for the pharmacological modulation of the PTP *in vivo*.

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[1] The abbreviations used are: PTP, permeability transition pore; CsA, cyclosporin A; MOPS, 4-morpholinepropanesulfonic acid; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenyl hydrazone; PhAsO, phenylarside oxide; $Ub_0$, ubiquinone 0; decyl-Ub, decylubiquinone; $Ub_5$, ubiquinone 5; MBz, 2-methoxy-1,4-benzoquinone; DBz, 2,3-dimethoxy-1,4-benzoquinone.


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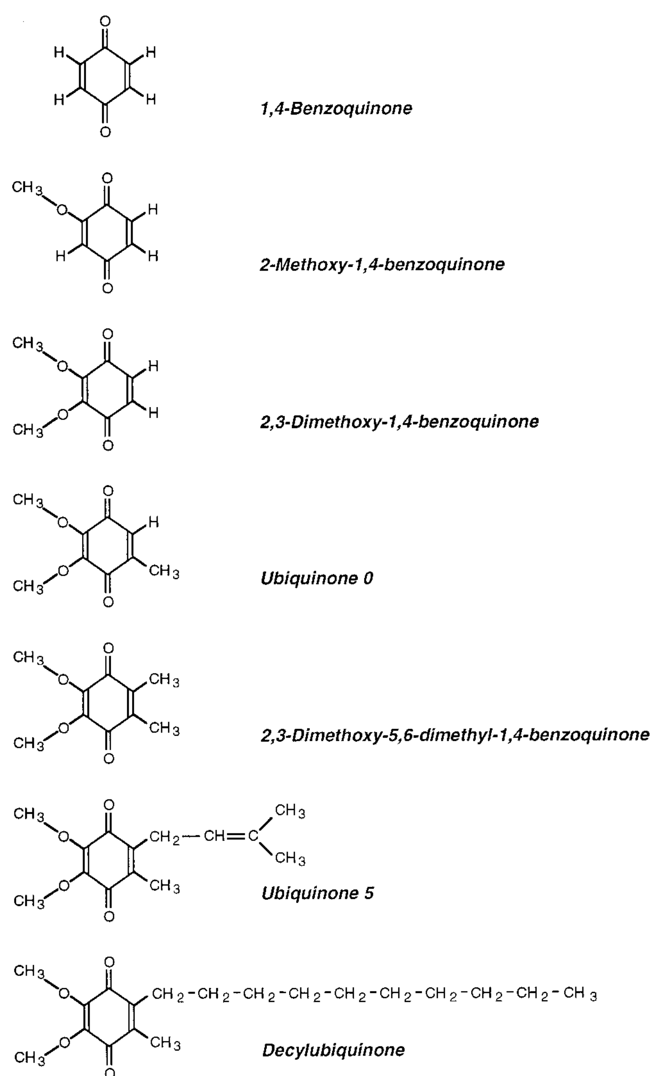


FIG. 1. **Chemical structure of the quinones tested in this study.**

## MATERIALS AND METHODS

Rat liver mitochondria were prepared according to standard differential centrifugation procedures in a medium containing 250 mM sucrose, 10 mM Tris-HCl (pH 7.4), and 0.1 mM EGTA-Tris.

Mitochondrial oxygen consumption was measured polarographically at 25 °C using a Clark-type oxygen electrode. Extramitochondrial $Ca^{2+}$ concentration was measured fluorimetrically in the presence of 1 $\mu$M Calcium Green-5N exactly as described in Ref. 16 (excitation-emission: 503–535 nm) with a thermostatted Perkin-Elmer LS-50B spectrofluorometer. Calibration of the signal was achieved by the addition of known amounts of $Ca^{2+}$. Mitochondrial volume changes were measured from the absorbance changes at 540 nm with a Perkin-Elmer Lambda 10 spectrophotometer equipped with magnetic stirring and thermostatic control.

$Ub_0$, $Ub_5$, decyl-Ub, and 1,4-benzoquinone were purchased from Sigma; MBz, DBz, and 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone were purchased from Apin Chemicals (Abingdon, UK); and CsA was a gift from Novartis (Basel, Switzerland). All other chemicals were of the highest purity commercially available. The chemical structure of the quinones tested in this work is presented in Fig. 1.

## RESULTS

*Effect of Quinones and CsA on Mitochondrial $Ca^{2+}$ Loading*—In the experiments depicted in Fig. 2, rat liver mitochondria energized with glutamate plus malate in the presence of 1 mM $P_i$ were loaded with a train of 25 $\mu$M $Ca^{2+}$ pulses at 1-min intervals. Under these conditions, mitochondria took up and retained $Ca^{2+}$ until the load reached a threshold of slightly less than 75 nmol of $Ca^{2+}$·mg protein$^{-1}$, a point at which mitochondria underwent a fast process of $Ca^{2+}$ release (*trace a*), which was accompanied by depolarization and swelling (not shown). The precipitous $Ca^{2+}$ release was due to the opening of the PTP, because the critical $Ca^{2+}$ load required to induce it was increased by CsA to above 325 nmol of $Ca^{2+}$·mg protein$^{-1}$ (*trace c*). This $Ca^{2+}$-loading protocol thus provides a convenient and sensitive assay of PTP inhibition or sensitization. When the quinones listed in Fig. 1 were tested, a striking PTP inhibition was observed specifically with $Ub_0$ (*trace f*) and decyl-Ub (*trace i*), whereas all other quinones were ineffective (*traces b, d, e, g,* and *h*). Based on the maximum $Ca^{2+}$-loading capacity, decyl-Ub was slightly less potent than CsA, whereas $Ub_0$ was more effective allowing mitochondria to reach a $Ca^{2+}$ load of about 500 nmol of $Ca^{2+}$·mg protein$^{-1}$.

The experiments shown in Fig. 3 report the concentration dependence of the effects of the active compounds, $Ub_0$ and decyl-Ub, on the $Ca^{2+}$ retention capacity of rat liver mitochondria energized with either succinate or glutamate and malate. It can be appreciated that both $Ub_0$ (*panel A*) and decyl-Ub (*panel B*) inhibited the PTP in a concentration-dependent fashion. $Ub_0$ was more effective when mitochondria were energized with glutamate plus malate (*open symbols*) than with succinate (*closed symbols*), whereas decyl-Ub displayed the opposite pattern. The optimal concentration for PTP inhibition was about 50 $\mu$M for $Ub_0$ (*panel A*) and 100 $\mu$M for decyl-Ub (*panel B*). Both $Ub_0$ and decyl-Ub became less efficient at higher concentrations, the drop being more pronounced when mitochondria were energized with glutamate and malate.

To explore the statistical significance of this finding, we measured the inhibitory effects of 50 $\mu$M $Ub_0$ and 100 $\mu$M decyl-Ub on a number of different mitochondrial preparations. Because the substrate dependence of the maximal mitochondrial $Ca^{2+}$ loading attainable with CsA has never been addressed, we also measured this parameter in the presence of 1 $\mu$M CsA. Fig. 4 shows that $Ub_0$ was approximately 70% more potent than CsA when mitochondria were energized with glutamate and malate ($p = 0.0001$, unpaired Student's $t$ test). On the other hand, $Ub_0$ was slightly but not significantly based on an unpaired Student's $t$ test, less potent than CsA when mitochondria were energized with succinate. Whatever the substrates used, decyl-Ub was approximately 30% less potent than CsA ($p < 0.005$, unpaired Student's $t$ test). It should be noted that although the inhibitory efficiency of $Ub_0$ was maximal with Complex I substrates, inhibition by CsA and decyl-Ub was more pronounced with succinate as the substrate.

*Effect of Quinones on PTP Opening Induced by $P_i$, FCCP, Atractylate, and PhAsO*—The next series of experiments was performed to test whether quinones are general inhibitors of the PTP. In these protocols, mitochondria were loaded with a small amount of $Ca^{2+}$ that did not open the PTP *per se*, followed by a variety of well characterized PTP triggering agents. Onset of the permeability transition was then monitored from the changes of absorbance at 540 nm, which reflect mitochondrial permeabilization to sucrose. Fig. 5 shows that swelling could be easily induced by the addition of $P_i$ (*panel A*), ruthenium red plus FCCP (*panel B*), atractylate (*panel C*), or PhAsO (*panel D*) (*traces a* in all panels). Swelling was due to PTP opening because, as expected, it could be inhibited by CsA (*traces c* in all panels). Strikingly, both $Ub_0$ (*traces f* in all panels) and decyl-Ub (*traces i* in all panels) inhibited the permeability transition in all cases, the former quinone being more potent. It is noteworthy that DBz, which had no effect on the $Ca^{2+}$ retention capacity (see Fig. 2, *trace e*), inhibited the permeability transition induced by depolarization (*panel B*, *trace e*) or by PhAsO (*panel D*, *trace e*) in about 50% of the mitochondria and

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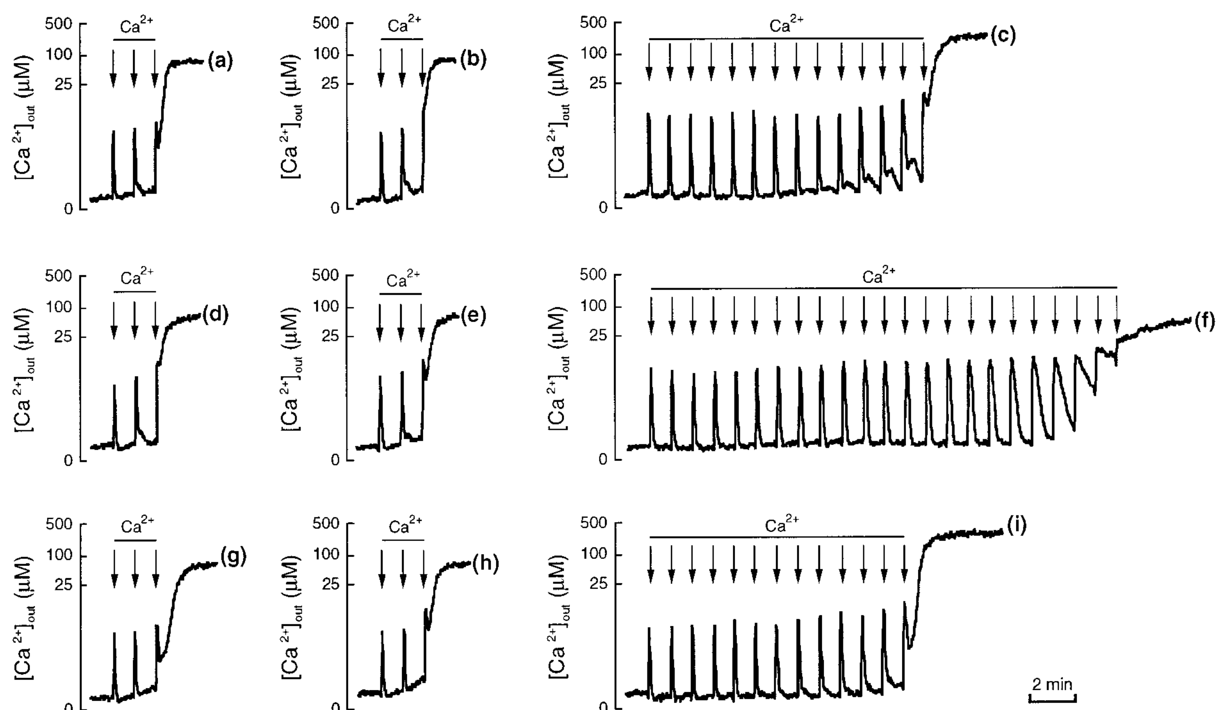


FIG. 2. **$Ca^{2+}$ retention capacity of rat liver mitochondria.** The effect of quinones and CsA is shown. The incubation medium contained 250 mM sucrose, 1 mM $P_i$-Tris, 10 mM Tris-MOPS, 5 mM glutamate-Tris, 2.5 mM malate-Tris, 1 μM Calcium Green-5N. The final volume was 2 ml, pH 7.4, 25 °C. Experiments were started by the addition of 2 mg of mitochondria (not shown) followed by the addition of 12.5 μM 1,4-benzoquinone (*trace b*), 1 μM CsA (*trace c*), 12.5 μM MBz (*trace d*), 12.5 μM DBz (*trace e*), 50 μM $Ub_0$ (*trace f*), 50 μM 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone (*trace g*), 50 μM $Ub_5$ (*trace h*), or 100 μM decyl-Ub (*trace i*). Only $Ca^{2+}$ was added in *trace a*. Where indicated, 25 μM $Ca^{2+}$ pulses were added (*arrows*).

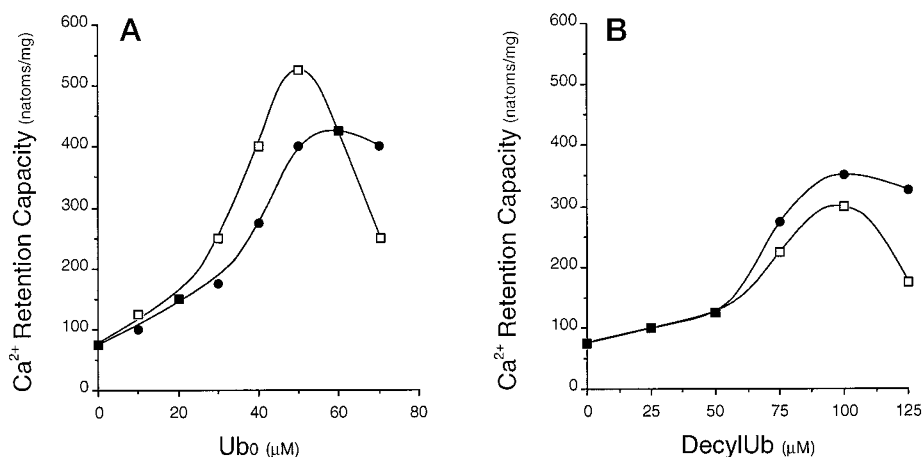


FIG. 3. **$Ca^{2+}$ retention capacity of rat liver mitochondria at increasing $Ub_0$ or decyl-Ub concentrations and the effect of respiratory substrates.** Experimental conditions were as in Fig. 2 except that the respiratory substrates were 5 mM glutamate-Tris and 2.5 mM malate-Tris (*open squares*) or 5 mM succinate-Tris (*closed circles*) in the presence of the indicated concentrations of $Ub_0$ (*panel A*) or decyl-Ub (*panel B*). Trains of $Ca^{2+}$ pulses at 1-min intervals were added exactly as shown in Fig. 2. The $Ca^{2+}$ retention capacity denotes the amount of $Ca^{2+}$ necessary to induce $Ca^{2+}$ release.

slightly delayed PTP opening induced by $P_i$ or atractylate (*trace e* in *panels A* and *C*, respectively). A delay in onset was also reproducibly observed with MBz when PTP was triggered by $P_i$ (*panel A*, *trace d*) or by PhAsO (*panel D*, *trace d*), whereas 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone (*trace g*) or $Ub_5$ (*trace h*) slightly but consistently increased the extent of swelling with all inducers.

*The PTP Inhibitory Effects of $Ub_0$ and decyl-Ub are Selectively Removed by $Ub_5$*—In the experiments depicted in Fig. 6, mitochondria energized with glutamate and malate in the presence of 1 mM $P_i$ were supplemented with CsA, $Ub_0$, or decyl-Ub and loaded with 100 nmol of $Ca^{2+}$·mg protein$^{-1}$, a load that opened the pore in the absence of inhibitors (results omitted for clarity). After 2 min, mitochondria were exposed to $Ub_5$, a ubiquinone that was unable to increase the $Ca^{2+}$ retention capacity (see Fig. 2). It can be appreciated that $Ub_5$ caused PTP opening in the presence of decyl-Ub or $Ub_0$ but not CsA (Fig. 6). Addition of rotenone with succinate as the substrate or antimycin A with ascorbate plus *N,N,N′,N′*-tetramethyl-1,4-phenylenediamine dihydrochloride as the substrate did not modify pore inhibition by $Ub_0$ or decyl-Ub (results not shown). Thus, $Ub_5$ specifically competed with the inhibitory quinones suggesting that: (i) inactive quinones may bind to the same site(s) as the active ones; and (ii) their binding site is separate from that of CsA and rotenone and antimycin A, which do not inhibit the pore *per se* (Refs. 25 and 26). These data indicate that some, but not all, ligands are able to stabilize the pore in the closed conformation.

*Effects of Quinones on Respiration*—It has been shown that short chain quinones, besides being electron acceptors, also inhibit Complex I activity (27, 28). Because this side effect could strongly limit the interest of these compounds as PTP modulators in living cells, we evaluated the effects of the quinones used in this study on the uncoupled respiration of rat liver mitochondria energized either with succinate or glutamate and malate. Fig. 7 shows that, with only the exception of

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decyl-Ub, all quinones strongly inhibited respiration with both Complex I and Complex II substrates (*open* and *closed symbols*, respectively). It should be noted that $Ub_0$ inhibited uncoupled respiration in the same range of concentrations effective at PTP opening. However, respiratory inhibition by $Ub_0$ was not complete, it did not affect state 4 respiration (not shown) and it did not interfere with the electrical charge compensation required for normal $Ca^{2+}$ uptake under our loading protocols (see Fig. 2). Finally, it should be noted that decyl-Ub had negligible effects on respiration supported by glutamate and malate and only a small inhibitory effect when succinate was the substrate.

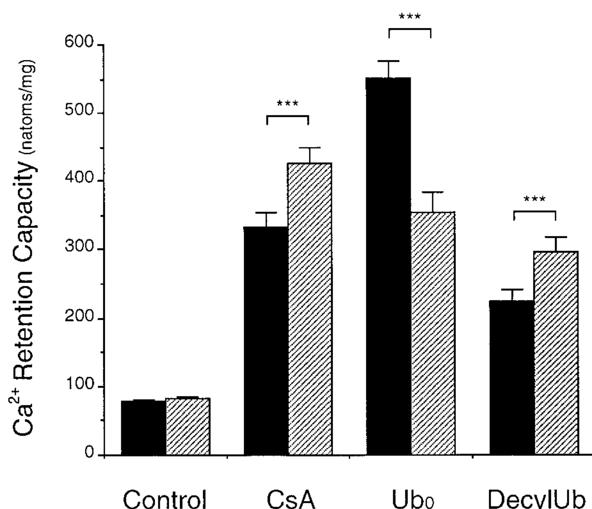


FIG. 4. **Comparison of the effect of CsA, $Ub_0$, and decyl-Ub on the mitochondrial $Ca^{2+}$ retention capacity with different substrates.** The mitochondrial $Ca^{2+}$ retention capacity was measured exactly as in Fig. 2 in the absence of inhibitors (control) or in the presence of 1 $\mu$M CsA, 50 $\mu$M $Ub_0$, or 100 $\mu$M decyl-Ub. Results are mean ± S.E. ($n$ = 21, 15, 6, and 8 for the control, CsA, $Ub_0$, and decyl-Ub, respectively). *Solid bar*, glutamate-malate; *hatched bar*, succinate; ***, $p < 0.005$, paired Student's $t$ test.

DISCUSSION

In this study we have shown that: (i) $Ub_0$ and decyl-Ub are general inhibitors of the PTP, because inhibition was observed irrespective of the method used to open the pore (Fig. 4); (ii) inhibitory quinones may act through a specific binding site, which is different from the CsA-binding site because the inhibitory effect could be *specifically* counteracted by pore-inactive quinones when the PTP was inhibited by $Ub_0$ or decyl-Ub but not by CsA (Fig. 5); and (iii) $Ub_0$ is most effective when Complex I substrates are oxidized, whereas decyl-Ub and CsA are most effective with succinate as the substrate (Fig. 3). Although understanding the *mechanism* of inhibition by quinones will require further testing of specific structural variants, we conclude that $Ub_0$ and decyl-Ub define a novel structural class of PTP modulators.

*Quinones Are General Inhibitors of the PTP*—$Ub_0$ and decyl-Ub inhibit the PTP irrespective of the method used to induce its opening, *i.e.* addition of $P_i$, depolarization with uncoupler, and addition of atractylate or PhAsO (Fig. 5), or *tert*-butylhydroperoxide with identical results (not shown). PhAsO acts at the level of a redox-sensitive dithiol that is the site of action of many oxidants (including *tert*-butylhydroperoxide) through oxidized glutathione (25, 26); this site can be selectively blocked by monobromobimane, which is not a general pore inhibitor (29). Atractylate induces the PTP through an undefined mechanism that may involve conformational changes of the adenine nucleotide translocase (30). Depolarization is likely to act through a postulated PTP voltage sensor (31) that can be blocked by arginine-selective reagents (32). $P_i$ is the most classical PTP inducer, and its effects on the pore can be discriminated from those of other PTP effectors (33). Because inhibitory quinones are able to prevent PTP opening in all of these cases we conclude that, like CsA, they act at a site that is downstream of the site of action of all inducers.

*Mechanism of PTP Modulation by Quinones*—Endogenous ubiquinone is the only lipidic constituent of the respiratory chain. It is involved in redox reactions in Complex III (Q cycle), and it acts as an electron shuttle between Complexes I, II, and III (see Ref. 34 for review). The total number of quinone-

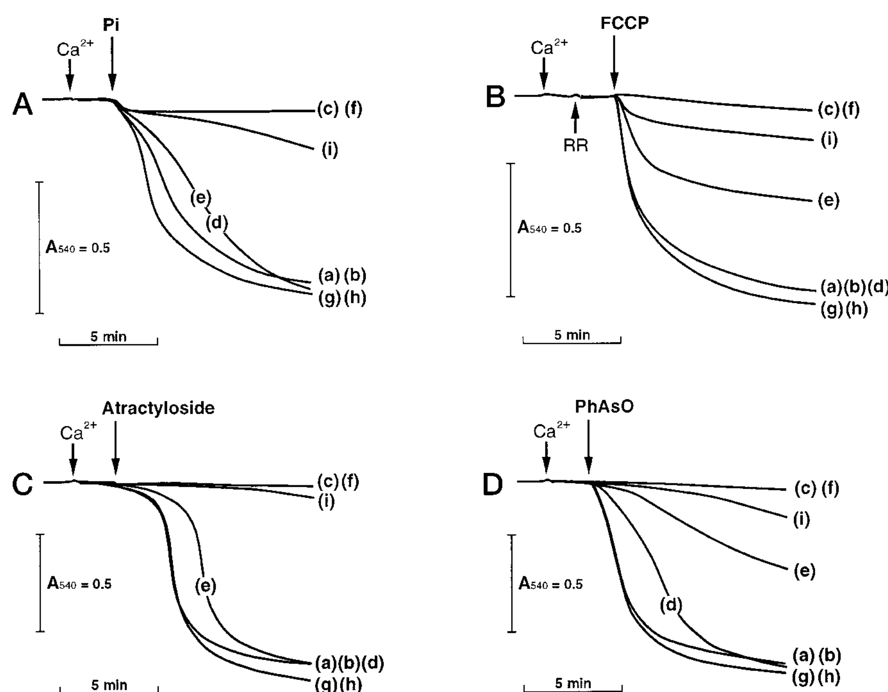


FIG. 5. **Effect of quinones and CsA on PTP opening induced by $P_i$, FCCP, atractylate, and PhAsO.** Experimental conditions were as in Fig. 2 except that Calcium Green-5N was omitted. PTP opening was monitored as the absorbance decrease at 540 nm. For all panels, the medium was supplemented with: *trace a,* no further additions; *trace b,* 12.5 $\mu$M 1,4-benzoquinone; *trace c*, 1 $\mu$M CsA; *trace d*, 12.5 $\mu$M MBz; *trace e,* 12.5 $\mu$M DBz; *trace f*, 50 $\mu$M $Ub_0$; *trace g*, 50 $\mu$M 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone; *trace h*, 50 $\mu$M $Ub_5$; and *trace i,* 75 $\mu$M decyl-Ub. Experiments were started by the addition of 2 mg of mitochondria (not shown). Where indicated, additions were 25 $\mu$M $Ca^{2+}$ and 20 mM $P_i$ (*panel A*); 20 $\mu$M $Ca^{2+}$, 1 $\mu$M rutheniun red (*RR*) and 200 nM FCCP (*panel B*); 25 $\mu$M $Ca^{2+}$ and 500 $\mu$M atractylate (*panel C*); 20 $\mu$M $Ca^{2+}$ and 25 $\mu$M PhAsO (*panel D*).

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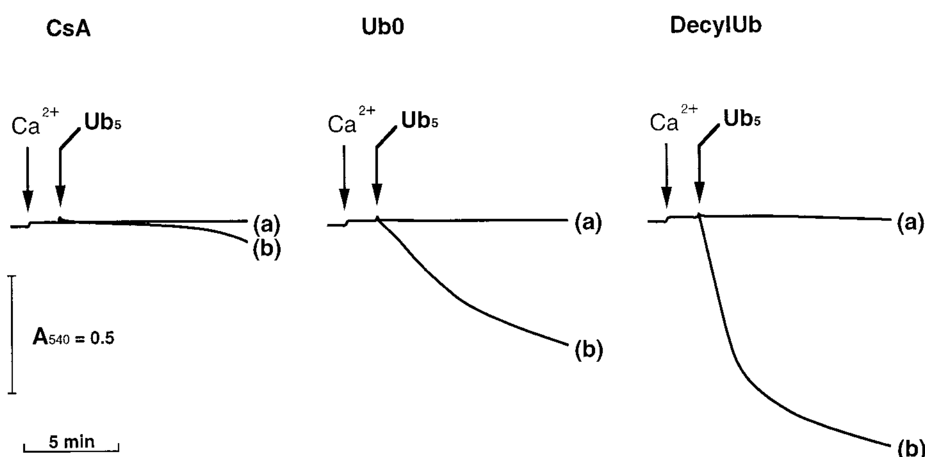


FIG. 6. **$Ub_5$ relieves inhibition of the PTP by $Ub_0$ and decyl-Ub but not CsA.** Experimental conditions were as in Fig. 5. Experiments were started by the addition of 2 mg of mitochondria (not shown) in the presence of 1 $\mu$M CsA, 50 $\mu$M $Ub_0$, or 75 $\mu$M decyl-Ub, as indicated on each trace. Where indicated, 100 $\mu$M $Ca^{2+}$ (all *traces*) and 200 $\mu$M $Ub_5$ (*traces b* only) were added.

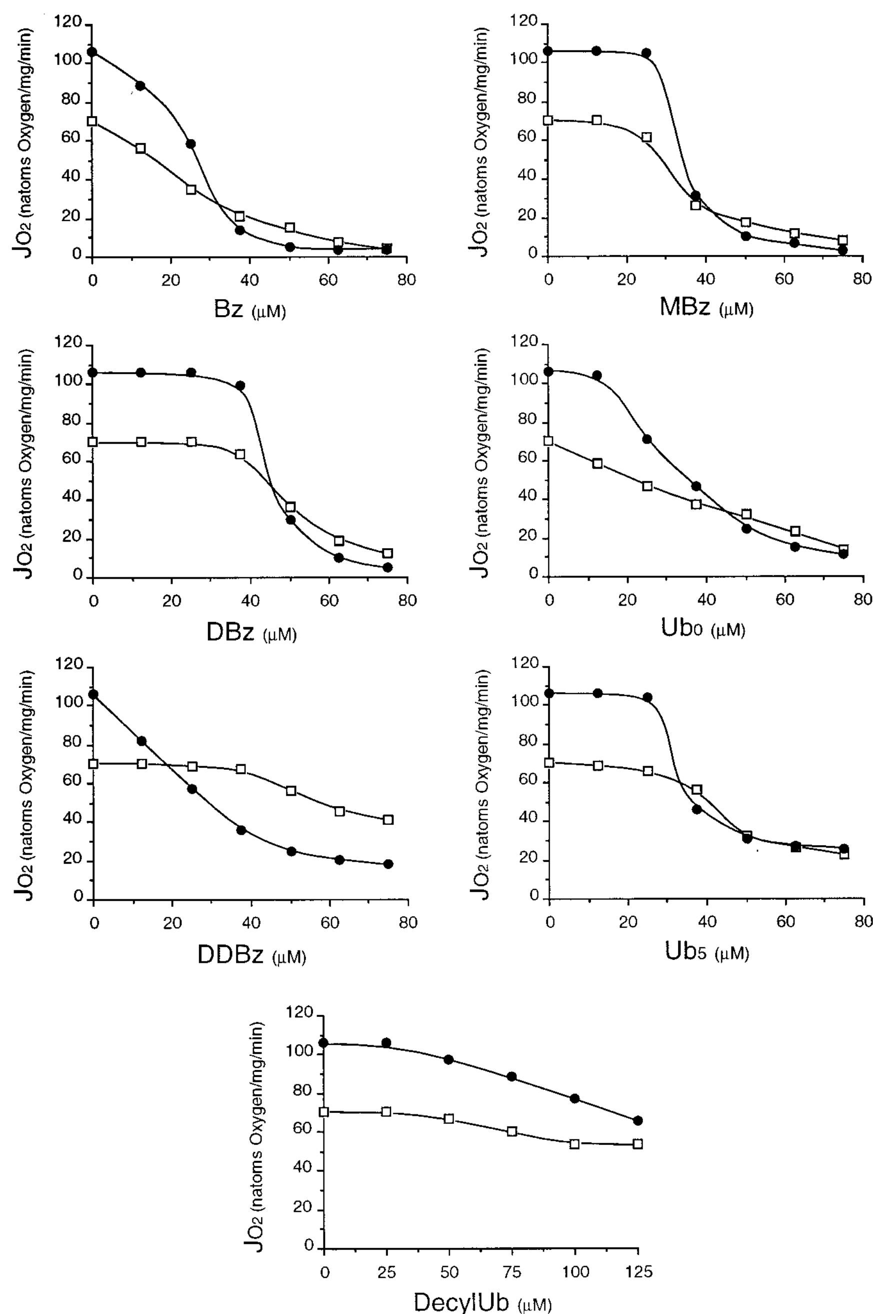


FIG. 7. **Effect of quinones on the maximal rates of respiration with different respiratory substrates.** Rat liver mitochondria (2 mg) were incubated in a medium containing 250 mM sucrose, 1 mM $P_i$-Tris, 10 mM Tris-MOPS, 100 $\mu$M EGTA, 1 $\mu$M CsA, 200 nM FCCP, and the indicated concentration of quinones (final volume, 2 ml; pH 7.4; 25 °C). The respiratory substrates were 5 mM glutamate-Tris and 2.5 mM malate-Tris (*open squares*) or 5 mM succinate-Tris (*closed circles*). *Bz*, 1,4-benzoquinone; *DDBz*, 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone.

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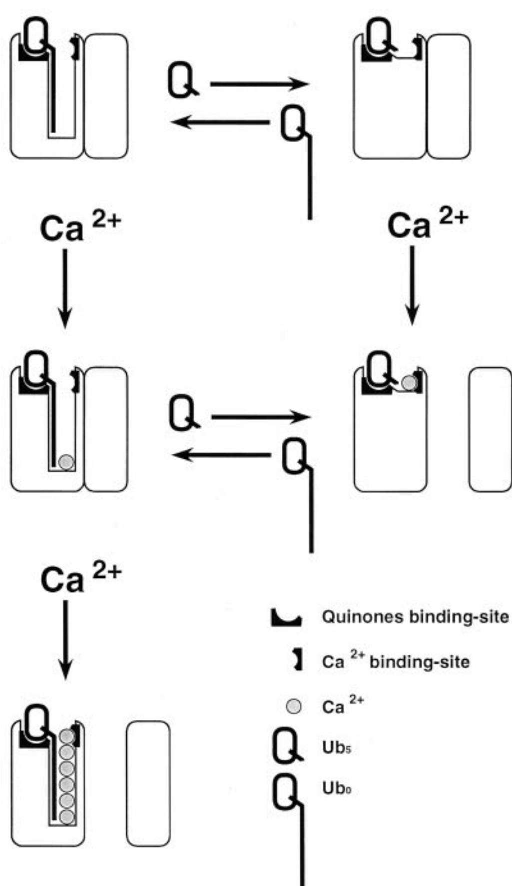


FIG. 8. **Model for PTP regulation by ubiquinones.** The *open space* between the *rectangles* denotes the open state of the PTP. For an explanation, see text.

binding sites in the respiratory chain has not been defined with certainty, but in the case of Complex I at least two binding sites have been identified (35, 36). All the quinones used in the present study clearly interfere with electron transfer (Fig. 7). On the other hand, only $Ub_0$ and decyl-Ub (the latter being a very weak respiratory inhibitor) are able to inhibit the PTP. Taken together, these observations demonstrate that PTP inhibition by quinones is not directly related to inhibition of respiration.

A study of PTP inhibition with the $Ca^{2+}$- and energy-dependent assays employed in this work cannot be carried out at quinone concentrations that inhibit $Ca^{2+}$ uptake. We found strong inhibition of $Ca^{2+}$ uptake at concentrations of 1,4-benzoquinone, MBz, and DBz higher than those employed in Fig. 2 (data not shown), and this poses an upper limit to the usable concentrations of these compounds. Thus, it remains possible that apparently PTP-inactive compounds have a lower affinity for the PTP-binding site(s). Yet we know with certainty that specific quinone structural features are required for PTP inhibition, because pore-inactive $Ub_5$ concentrations specifically relieve inhibition by $Ub_0$ and decyl-Ub (Fig. 6). This is a critical result that can be most easily explained by postulating the existence of a quinone-binding site on the pore, which would be shared by $Ub_0$, decyl-Ub, and $Ub_5$.

The existence of inactive ligands, such as $Ub_5$, suggests that the interaction of quinones induces PTP conformational changes that are ligand-specific, and we suspect that endogenous quinones are pore ligands that stabilize it in the closed conformation. In this context, the addition of inhibitory quinones would increase the stability of the pore closed conformation, suggesting that not all of the binding sites are saturated by endogenous quinones. Addition of inactive ligands, such as $Ub_5$, would first saturate these sites and then displace endogenous quinones, thus increasing the pore open probability. Consistent with this idea, concentrations of $Ub_5$ higher than 200 $\mu$M slightly decreased the $Ca^{2+}$ retention capacity (results not shown).

The scheme of Fig. 8 depicts our current model to explain the effects of quinones that inhibit the PTP (exemplified here by $Ub_0$) and quinones that specifically relieve this inhibition (exemplified here by $Ub_5$). In the closed state, the pore can exist in the $Ub_0$- or $Ub_5$-liganded state, which confer different conformations to the pore resulting in a different accessibility to $Ca^{2+}$. These conformations are in equilibrium according to the relative membrane concentration of ubiquinones and to their binding affinities, which remain undefined. Addition of a limited $Ca^{2+}$ load can only open the PTP in the $Ub_5$-liganded conformation, whereas a higher $Ca^{2+}$ load is required to access the $Ca^{2+}$-binding site(s) in the $Ub_0$-liganded conformation. Thus, PTP opening can be achieved by either increasing the $Ca^{2+}$ load or by displacing the inhibitory quinone. We consider this a simple working hypothesis that is consistent with all the experimental results of the present paper.

*Regulation of PTP by Electron Flux at Complex I*—In a previous study (24), we have shown that electron flux through respiratory chain Complex I is an important PTP regulator, both in skeletal muscle and in liver mitochondria, in the sense that increased electron flux through Complex I favors PTP opening at a given $Ca^{2+}$ load. We believe that the scheme of Fig. 8 easily accommodates these observations as well. Indeed, if endogenous quinones bind the PTP stabilizing it in the closed conformation, increasing electron flux within Complex I may displace quinones from this site increasing in turn the pore open probability.

This interpretation is consistent with another set of observations described in the present paper. CsA and $Ub_0$ inhibit PTP opening with comparable efficiency when succinate is the respiratory substrate, a condition where electron flux through Complex I is extremely slow and has probably no influence on the PTP regulation. On the other hand, when Complex I is turning over, $Ub_0$ becomes more potent than CsA (Figs. 3 and 4). The data suggest that $Ub_0$ may rapidly bind to sites liberated by increased electron flux and previously occupied by endogenous quinones and that $Ub_0$ is intrinsically more potent than endogenous quinones as a PTP inhibitor.

We note that pore regulation by Complex I activity in liver has been generally underestimated because it strongly depends on the incubation conditions, such as the presence of $P_i$ and $Mg^{2+}$ (24). Furthermore, the most common energization condition in studies with isolated mitochondria is the combination of succinate and rotenone, a situation where any contribution coming from electron flux at Complex I cannot be observed by definition.

*Perspectives*—The molecular nature of the PTP remains unsolved, but the findings of the present work indicate that the PTP must have a quinone-binding site. Moreover, the pore is regulated by pyridine nucleotides, which induce major conformational changes on Complex I (37), and by the transmembrane potential (38). Taken together, these data suggest that Complex I may be a structural constituent of the PTP. Development and screening of high affinity, nontoxic quinones is in rapid progress in our laboratory. The synthesis of proper photoactive quinone derivatives should be instrumental in the molecular identification of the quinone-binding component(s) of the PTP.

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