

Singlet Oxygen Produced by Photodynamic Action Causes Inactivation of the Mitochondrial Permeability Transition Pore*

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We have studied the effects of singlet oxygen produced by photodynamic action on the cyclosporin A-sensitive permeability transition (PT) in isolated rat liver mitochondria. Mitochondria were incubated with 3 μ M hematoporphyrin and irradiated at 365 nm with a fluence rate of 25 watts/m². For short durations of irradiation (60 s) the adenine nucleotide translocase was inactivated, but mitochondria retained their ability to form a proton electrochemical gradient and accumulated Ca²⁺ and P_i at the same rate as non-irradiated controls. Strikingly, however, the oxidative effects of photodynamic action prevented opening of the PT pore which is normally induced by Ca²⁺ plus P_i or by treatment with diethyl pyrocarbonate (a histidine reagent) or diamide (a thiol oxidant). We show that the most likely targets for photodynamic action are critical histidines that undergo degradation. Irradiated, hematoporphyrin-loaded mitochondria treated with diethyl pyrocarbonate or diamide still undergo the PT when treated with phenylarsine oxide, which reacts with a critical dithiol involved in pore modulation (Petronilli, V., Costantini, P., Scorrano, L., Colonna, R., Passamonti, S., and Bernardi, P. (1994) *J. Biol. Chem.* 269, 16638–16642). These data suggest (i) that the dithiol cysteines are not oxidized by photodynamic action, but rather became inaccessible to oxidants; and (ii) that irradiation of hematoporphyrin-loaded mitochondria does not lead to pore denaturation, but rather to site-selective inactivation of discrete pore functional domains.

When Ca²⁺-loaded mitochondria are treated with a variety of inducing agents, the opening of Ca²⁺-dependent pores leads to the so-called permeability transition of the inner membrane, which is specifically inhibited by nanomolar concentrations of cyclosporin A. Among Ca²⁺-dependent pore inducers, oxidizing agents have received considerable attention, and redox state changes of pyridine nucleotides, glutathione, or sulfhydryl groups have been shown to have a prominent role in the mechanism (or in the control) of Ca²⁺ efflux following pore opening (for general reviews, see Refs. 1 and 2).

In the present work, we have studied the effects of the very

reactive species, singlet oxygen, ¹O₂, on isolated rat liver mitochondria. The presence of ¹O₂ as a transient species able to damage cells, mainly at the mitochondrial level, can be observed in disease, such as in porphyria (3), and in the treatment of solid tumor by porphyrin photodynamic therapy (4). The effects of ¹O₂ on substrate transport, respiration, and phosphorylation are well documented, but few reports deal with those on the uptake and release of Ca²⁺ from mitochondria (for a general review, see Ref. 5). In 1981 we have reported (6) that treatment with ¹O₂ produced by porphyrin photodynamic action can prevent Ca²⁺ release due to membrane "damage" resulting from massive Ca²⁺ loading. At that time, our interpretation was that such a release from Ca²⁺-overloaded mitochondria was probably of no physiological relevance. This aspect must now be reconsidered in the light of recent developments in the mitochondrial PT¹ field. It is now widely accepted that Ca²⁺ release can occur through opening of a specific channel sensitive to cyclosporin A rather than to unspecific membrane damage. These advances prompted us to study more thoroughly the effect of photodynamic action on mitochondrial energy transduction and Ca²⁺ fluxes. As in our previous studies, HP has been chosen as an exogenous photosensitizer in isolated rat liver mitochondria. Our results show that, in sharp contrast with other activated oxygen species such as H₂O₂ or O₂⁻ (1), ¹O₂ does not induce PT. Rather HP photodynamic action leads to suppression of the PT induced by Ca²⁺ plus P_i, DEPC, or DIA.

Photoirradiated, HP-loaded mitochondria treated with DEPC or DIA still undergo the PT when treated with PhAsO, which reacts with a critical dithiol involved in pore modulation (7). These data indicate that the dithiol cysteines are not oxidized by ¹O₂, but rather became inaccessible to oxidants, and suggest that irradiation of HP-loaded mitochondria does not lead to pore denaturation, but rather to site-selective inactivation of discrete pore functional domains comprising critical histidines.

MATERIALS AND METHODS

Preparation of rat liver mitochondria was performed as described previously (8). To compare with maximum accuracy the various responses, mitochondria were placed in a vessel fitted with both an oxygen and a Ca²⁺ electrode (9), and all the irradiations were performed under the same optical conditions, *i.e.* 3 μ M HP, giving an absorbance of 0.14/cm at the irradiation wavelength of 365 nm. Irradiation was performed using a Philips HPW 125-watt lamp (Philips, Eindhoven, The Netherlands) after 120 s incubation in the dark, since uptake of HP in

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¹ The abbreviations used are: PT, permeability transition; HP, hematoporphyrin IX; DEPC, diethyl pyrocarbonate; DIA, diamide; AdNT, adenine nucleotide translocase; PhAsO, phenylarsine oxide; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenyl hydrazone; Mops, 4-morpholinopropanesulfonic acid.

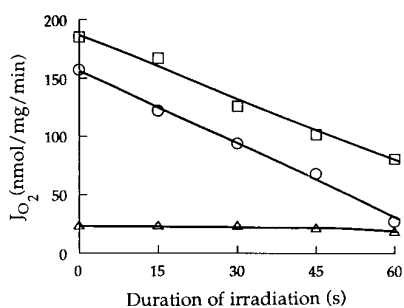


FIG. 1. **Effect of HP + light on respiration.** Mitochondria (0.5 mg/ml) were suspended in a medium containing 24 mM glycylglycine, 10 mM $MgCl_2$, 60 mM KCl, 7 mM KH_2PO_4 , 87 mM sucrose, pH 7.4, 14 mM succinate, and 2 μ g/ml rotenone) in the presence of 3 μ M HP in a thermostated ($T = 25^\circ C$) water-jacketed vessel. The O_2 consumption was followed using a Clark electrode connected to a recorder. After 120 s incubation, the suspension was irradiated. Respiration of mitochondria in state 4 (Δ), in state 3 (\circ) (1 mM ADP), or uncoupled (\square) (1.30 μ M FCCP) is plotted *versus* the duration of irradiation.

isolated mitochondria reaches a plateau within 120 s (10). The fluence rate incident to the mitochondrial suspension was 25 watts/ m^2 , as measured with a Black Ray UV meter (J 221, Ultraviolet Products, Inc., San Gabriel, CA). All irradiations were performed at 25 $^\circ C$ under magnetic stirring. Proper controls were carried out, indicating that the inhibitors used (rotenone, oligomycin, and cyclosporin A) gave a negligible light absorption at the wavelength of irradiation and that neither photosensitizer addition in the dark nor illumination alone were able to produce any change in the measured parameters (results not shown).

P_i carrier activity was followed using the classical swelling technique of Chappel and Haarhoff (11).

Measurement of ADP/ATP exchange was performed fluorometrically in control and photosensitized mitochondria as described by Passarella *et al.* (12).

Mitochondrial swelling was followed spectroscopically as the decrease in the absorbance of the mitochondrial suspension at 540 nm measured with a SLM Aminco DW 2000 spectrophotometer operated in the split beam mode (13).

All reagents were of the finest available grade and were used without further purification. HP was purchased from Aldrich and 1 mM stock solutions were prepared in dimethyl sulfoxide. Cyclosporin A, a gift from Dr. A. Roche of the Laboratoires Sandoz, Rueil Malmaison, France, was dissolved in ethanol. Incubation conditions and further experimental details are given in the figure legends.

RESULTS

Effects of HP Photodynamic Action on Respiration and AdNT Translocator Activity—We have long been interested in the relative effects of irradiation on specific mitochondrial functions. We have shown that at increasing doses of radiation, oxidative phosphorylation is the first function to be lost, whereas respiration and Ca^{2+} cycling are more resistant (9). These results suggest that selective targets for the photodynamic effect of porphyrins exist in mitochondria and that these may be exploited to understand the mechanism(s) of phototoxicity.

Fig. 1 shows the effects of increasing durations of irradiation in the presence of 3 μ M HP on respiration in state 4 (in the absence of ADP) or 3 (in the presence of ADP) and after addition of FCCP. Short exposures to irradiation (up to 60 s) did not affect state 4 respiration, and ADP-stimulated respiration was more sensitive to photodynamic action than FCCP-uncoupled respiration. This suggests that neither electron transport nor membrane potential is severely impaired after short durations of irradiation, in good agreement with Ref. 14. Fig. 2 shows that in fact damage to the AdNT (rather than damage to the ATP-synthase, as indicated before in Ref. 14) is responsible for the inhibition of the oxidative phosphorylation. Indeed, the exchange rate as a function of ADP concentration at increasing irradiation times indicates a strong non-competitive inhibition of the carrier. From this set of experiments, we conclude that

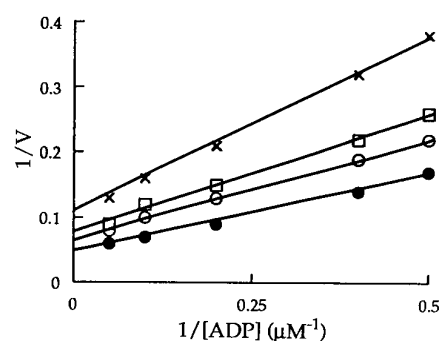


FIG. 2. **Effects of HP + light on the AdNT activity.** Experimental conditions were as in Fig. 1. After irradiation for various times, measurement of ADP/ATP efflux was performed in control mitochondria (\bullet), and in mitochondria irradiated for 60 s (\circ), 120 s (\square), or 240 s (\times) using an ATP-detecting system, exactly as described in Ref. 12.

the AdNT is a sensitive target that is easily inactivated by HP photodynamic action.

Effects of HP Photodynamic Action on Mitochondrial Ca^{2+} Fluxes—As indicated above, Ca^{2+} cycling in isolated mitochondria appears to be less sensitive to photodynamic action than oxidative phosphorylation (9). In this paper, we have specifically addressed the issue of how irradiation affects Ca^{2+} uptake and release from mitochondria and the pathways for Ca^{2+} efflux under these conditions.

To study the effects of irradiation on Ca^{2+} transport, Ca^{2+} uptake was measured after various periods of irradiation and recorded as the initial rate of Ca^{2+} influx in mitochondria energized with succinate + rotenone under conditions where Ca^{2+} uptake is not limited by the rate of respiration (20 μ M $CaCl_2$ in the presence of 10 mM Mg^{2+} , see Ref. 15). Fig. 3 shows that the initial rate of Ca^{2+} uptake strongly declined only after 180 s of irradiation. This is consistent with the results of Fig. 1, indicating that brief irradiations leave mitochondria with a good respiratory capacity, used here to drive Ca^{2+} uptake along the electrochemical gradient. Inhibition of Ca^{2+} transport was not a consequence of inactivation of the P_i carrier, since up to 240 s of irradiation had negligible effects on the rate of P_i uptake measured according to Ref. 11 (data not shown).

Fig. 4 shows a typical experiment where mitochondria energized with succinate + rotenone, and loaded with 80 μ M Ca^{2+} , were subsequently exposed to continuous irradiation. Initially, mitochondria treated in this way remained in a steady state, maintaining an external Ca^{2+} concentration of about 1 μ M. After over 120 s of irradiation, a process of Ca^{2+} efflux ensued, which could not be inhibited by the addition of 0.8 μ M cyclosporin A to the medium. We conclude that long durations of irradiation (>120 s) inhibit respiration to the point that the proton electrochemical gradient can no longer be maintained. Under these conditions Ca^{2+} leaves the matrix by reverse operation of the uniporter rather than through the PT. Shorter irradiation times, on the other hand, do not impair Ca^{2+} uptake or retention by mitochondria (Figs. 3 and 4). The latter condition was then exploited to test the effect(s) of photodynamic action on the PT.

Effects of HP Photodynamic Action on the Formation of PT Pores—The experiment of Fig. 5 illustrates the effect of increasing irradiation duration on the ability of HP-treated mitochondria to take up and retain Ca^{2+} . Non-irradiated mitochondria did not retain a 150 μ M Ca^{2+} pulse, and a fast process of Ca^{2+} release readily followed the initial phase of Ca^{2+} uptake (trace A) because of a PT (see Fig. 6). After 30 s of irradiation, mitochondria readily accumulated the initial 150 μ M Ca^{2+} pulse (trace B), whereas Ca^{2+} efflux was triggered by a further 20 μ M Ca^{2+} load (trace C). After 60 s of irradiation the

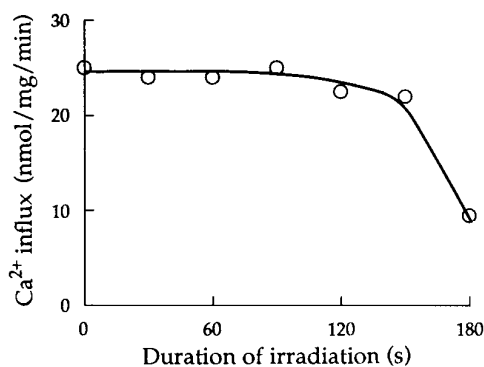


FIG. 3. Effects of HP + light on the rate of Ca^{2+} influx in mitochondria. Experimental conditions were as in Fig. 1. Ca^{2+} movements were followed using a Ca^{2+} -selective electrode (Radiometer F 2110 calcium electrode, Copenhagen, Denmark) inserted into the reaction chamber. After irradiation for various times, $20 \mu M$ Ca^{2+} was added to the medium. Ca^{2+} uptake was measured as the initial rate of Ca^{2+} influx in nmol/mg/min.

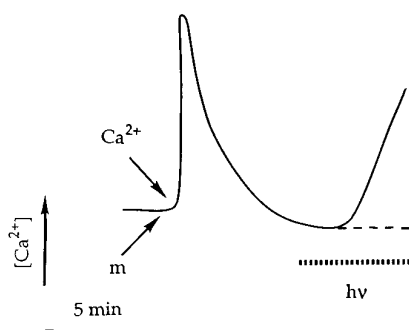


FIG. 4. Effects of HP + light on mitochondria loaded with Ca^{2+} before irradiation. Experimental conditions were as in Fig. 3. Mitochondria (*m*) were loaded with $80 \mu M$ Ca^{2+} and continuously irradiated (*hν*). The same trace was obtained in the presence of $0.8 \mu M$ cyclosporin (data not shown). Dashed trace represents non-irradiated mitochondria.

ability of mitochondria to retain Ca^{2+} was further improved (trace C), whereas higher irradiation periods impaired the initial rate of Ca^{2+} uptake (trace D), as expected from the extent of respiratory inhibition and therefore of impairment of membrane potential regeneration (Figs. 3 and 4). For this reason, 60 s of irradiation were used in all subsequent experiments.

The experiments of Fig. 6 were designed to test the pathways for Ca^{2+} efflux (followed by a Ca^{2+} -selective electrode) and the occurrence of a PT (followed spectroscopically) in sucrose-based media. Fig. 6, panel A, shows that non-irradiated mitochondria underwent a fast, spontaneous Ca^{2+} release process when challenged with a $150 \mu M$ Ca^{2+} pulse (trace a), which could be totally prevented by 800 nM cyclosporin A (not shown). As expected, spontaneous Ca^{2+} efflux was prevented by 60 s of irradiation, yet Ca^{2+} efflux was readily observed after the addition of the uncoupler FCCP (trace b). This process of Ca^{2+} efflux was not prevented by cyclosporin A (trace c), indicating that upon addition of FCCP rapid reversal of the uniport takes place. The experiments reported in Fig. 6, panel B, show that non-irradiated mitochondria were permeabilized to sucrose by Ca^{2+} (trace a), whereas irradiated mitochondria maintained their permeability barrier when challenged with an identical Ca^{2+} load (trace b), and yet underwent permeabilization and swelling upon addition of FCCP (trace b), in a process that maintained full sensitivity to cyclosporin A (trace c). These findings represent a first indication that the PT pores can still form and open upon depolarization even in photoirradiated mitochondria.

Reversible protonation of histidyl residues and oxidation of thiol groups play an important role in the modulation of

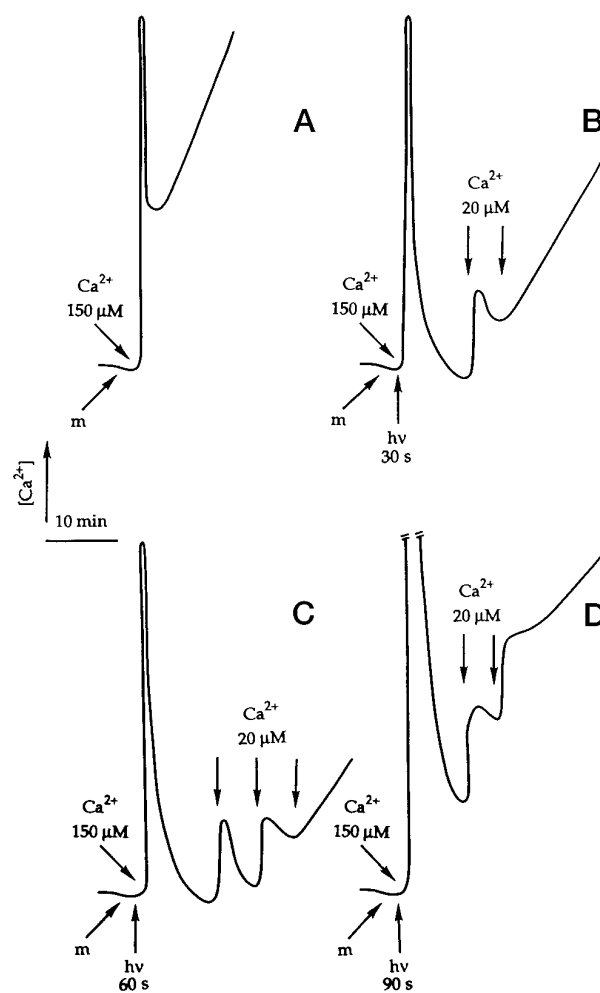


FIG. 5. Ca^{2+} loading of mitochondria following a preliminary irradiation with various doses of light. Experimental conditions were as in Fig. 3. Non-irradiated mitochondria loaded with $150 \mu M$ Ca^{2+} (arrows) (A) and mitochondria irradiated for 30 s (B), 60 s (C) or 90 s (D) were loaded immediately after irradiation with $150 \mu M$ Ca^{2+} (arrows), followed by additions of $20 \mu M$ Ca^{2+} where indicated (arrows).

pore opening (2). As 1O_2 is very reactive with histidine and thiol groups (16), we have determined whether inducers specifically reacting with these structures have a different mode of action after irradiation. Using the same swelling technique, PT was studied in mitochondria loaded with $40 \mu M$ Ca^{2+} .

Fig. 7, panel A, shows that addition of DIA to control mitochondria induced the expected PT, with rapid permeabilization to sucrose after a short lag phase (trace a). Strikingly, however, DIA was not able to cause pore opening in irradiated mitochondria (trace b), suggesting that the pore can no longer be induced by thiol oxidation. In irradiated mitochondria the pore could still be opened by the hydrophobic dithiol reagent, PhAsO (trace c), indicating that the dithiol cysteines have not undergone oxidation but are rather not accessible to oxidants from the aqueous phase. It should be mentioned that addition of PhAsO alone in these protocols was followed by pore opening (not shown). Finally, the lack of pore opening by DIA could be observed in a range of concentrations that are very effective at pore opening in control mitochondria (Fig. 7, panel B).

Fig. 8, panel A, reports the results of a similar experiment where control or irradiated mitochondria were treated with DEPC. As expected (17) DEPC treatment caused pore opening in control mitochondria (trace a). On the other hand, DEPC was unable to cause a PT in irradiated mitochondria (trace b), suggesting that histidine(s) are among the targets of 1O_2 . Also

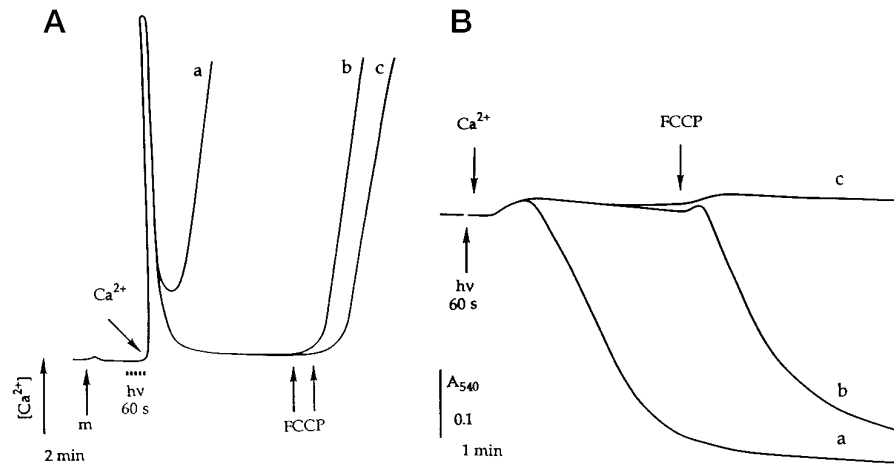


FIG. 6. Effects of HP + light on mitochondrial Ca^{2+} fluxes and on permeabilization to sucrose. The incubation medium contained 200 mM sucrose, 10 mM Tris-Mops, pH 7.4, 5 mM succinate Tris, 1 mM P_i , 10 μ M EGTA-Tris, 2 μ g/ml rotenone, and 3 μ g/ml oligomycin. Panel A, Ca^{2+} efflux measured with a Ca^{2+} -selective electrode. Where indicated (arrows) 0.5 mg/ml mitochondria (m), 150 μ M Ca^{2+} , and 0.2 μ M FCCP were added. Trace a, control mitochondria; trace b, mitochondria irradiated for 60 s ($h\nu$) in the presence of 3 μ M HP before Ca^{2+} loading; trace c, mitochondria irradiated as in trace b in the presence of 0.8 μ M cyclosporin A. Panel B, PT formation followed spectroscopically. The experiments were started by addition of 0.5 mg/ml mitochondria (not shown). Trace a, control mitochondria; trace b, mitochondria irradiated for 60 s ($h\nu$) in the presence of 3 μ M HP before Ca^{2+} loading; trace c, control and mitochondria irradiated as in trace b in the presence of 0.8 μ M cyclosporin A. Where indicated (arrows), 150 μ M Ca^{2+} and 0.2 μ M FCCP were added.

FIG. 7. Effect of DIA on PT in mitochondria treated with HP + light. Experimental conditions are as in Fig. 6, panel B. Panel A: trace a, control mitochondria; traces b and c, mitochondria irradiated for 60 s in the presence of 3 μ M HP. Where indicated (arrows) 40 μ M Ca^{2+} , 100 μ M DIA, and (trace c only) 10 μ M PhAsO were added. Panel B, rate of mitochondrial permeabilization ($\Delta A/min$) as a function of the concentration of DIA under the same experimental conditions shown in panel A. Open symbols, irradiated mitochondria; closed symbols, non-irradiated mitochondria.

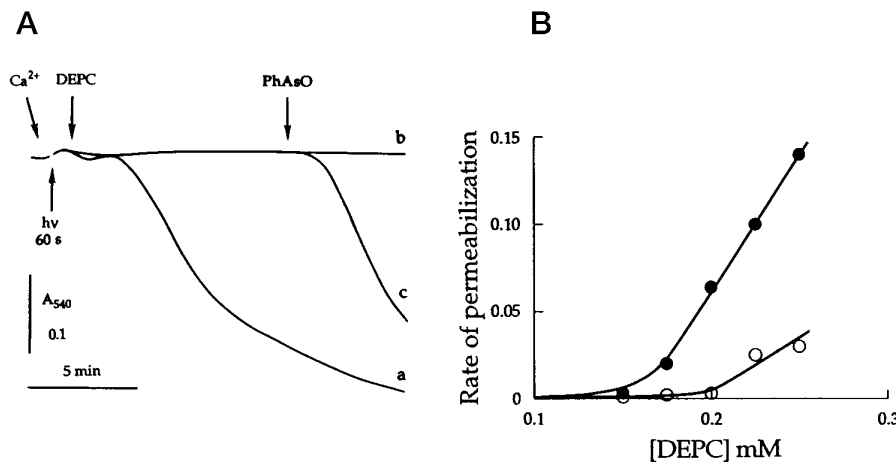
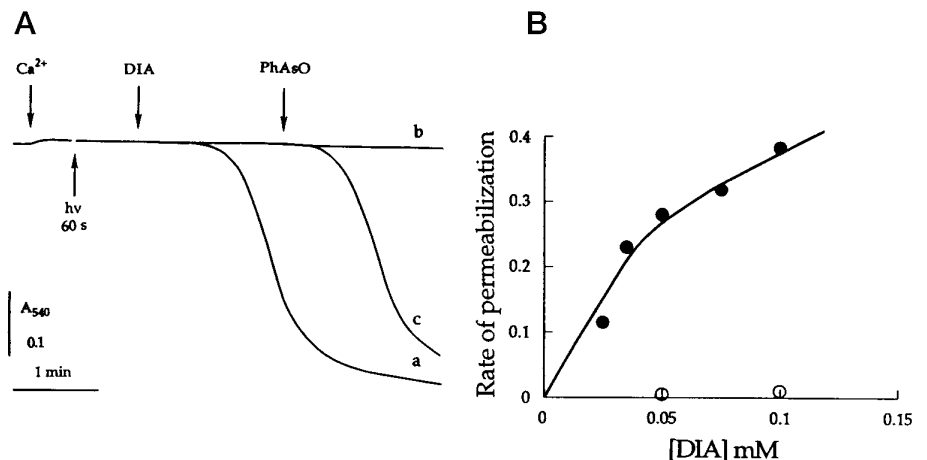


FIG. 8. Effect of DEPC on PT in mitochondria treated with HP + light. Experimental conditions were as in Fig. 6, panel B, except that P_i was omitted. Panel A: trace a, control mitochondria; traces b and c, mitochondria irradiated for 60 s in the presence of 3 μ M HP. Where indicated (arrows) 40 μ M Ca^{2+} , 200 μ M DEPC, and (trace c only) 10 μ M PhAsO were added. Panel B, rate of mitochondrial permeabilization ($\Delta A/min$) as a function of the concentration of DEPC under the same experimental conditions shown in panel A. Open symbols, irradiated mitochondria; closed symbols, non-irradiated mitochondria.

in this case the pore remained competent for opening, as demonstrated by the rapid permeabilization following the addition of PhAsO (trace c). Fig. 8, panel B, shows the effects of increasing concentrations of DEPC on pore opening in control (closed symbols) and irradiated mitochondria (open symbols). It can be appreciated that at concentrations higher than 0.2 mM DEPC the pore slowly opened even in irradiated mitochondria. This

effect, however, can be easily accounted for by respiratory inhibition, which in turn causes pore opening because of membrane depolarization (not shown, but see Ref. 17).

DISCUSSION

General Considerations—We have described the effects of irradiation on the mitochondrial PT in HP-treated mitochon-

dria, a well-defined system where the highly reactive species, 1O_2 is generated. We have shown that irradiation does not *cause* pore opening. Rather, irradiated HP-treated mitochondria no longer undergo a PT when challenged with Ca^{2+} plus P_i , DIA, or DEPC (Figs. 6–8). The experimental conditions (HP concentration, wavelength of irradiation, power density, duration of irradiation, and Ca^{2+} load) were carefully selected to minimize the effects of irradiation on the basic parameters modulating pore activity. In particular, (i) the respiratory capacity and the coupling efficiency of irradiated mitochondria were sufficient to form and maintain a proton electrochemical gradient (see also Ref. 18), as indicated by the maintenance of respiratory control with FCCP (Fig. 1); furthermore, a decreased proton electrochemical gradient should have *promoted* rather than inhibited the PT (2) and cannot therefore account for the present observations of pore inactivation. (ii) The steady-state Ca^{2+} accumulation maintained by irradiated mitochondria was identical to that maintained by the controls (Figs. 3 and 4), and the P_i carrier was negligibly affected by irradiation in the presence of HP (not shown); thus, pore inhibition cannot be explained by a lower Ca^{2+} load nor by a different response of matrix pH to Ca^{2+} uptake and release because of impaired P_i equilibration. (iii) Even if we cannot exclude a minute generation of O_2^- due to either energy transfers from HP excited triplet state (19) or increases in the respiratory chain e^- leakage after irradiation (20), ample evidence (reviewed in Refs. 1 and 2) indicates that reactive oxygen species *promote* the PT, most likely through oxidation of matrix glutathione (21–23); therefore, it is unlikely that pore inhibition is mediated by other activated oxygen species, which would rather counteract the inactivating effects of 1O_2 . From these considerations we conclude that pore inhibition in irradiated, HP-treated mitochondria most likely depends on *direct* effects of 1O_2 on critical residues important for pore function. Irrespective of the detailed mechanism(s) by which irradiation interferes with the pore, our data are consistent with the idea that 1O_2 selectively inactivates specific domains, either on the pore itself or on pore regulatory protein(s), normally acting as sensors for modulatory signals. Indeed, the effects of irradiation cannot be ascribed to a denaturation of the pore, since a PT can still be observed in irradiated mitochondria upon addition of FCCP after Ca^{2+} plus P_i loading (Fig. 6) or upon addition of PhAsO after treatment with DIA or with DEPC (Figs. 7 and 8).

Targets for 1O_2 — 1O_2 is a very reactive, transient species capable of damaging nucleic acids, proteins, and lipids (16). Its lifetime is very short in biological materials, which restricts its action to the close vicinity ($<0.1 \mu\text{m}$) of the photosensitizer (24). Although the membrane lipid phase is clearly involved in modulating the PT, possibly through surface potential effects (25, 26), we tend to exclude that pore inactivation by HP photodynamic action is due to lipid peroxidation, which should rather promote the PT (1).

The most likely target for 1O_2 -dependent pore inhibition appears to be proteinaceous in nature. HP dissolved in aqueous media preferentially accumulates in protein-binding sites of the inner mitochondrial membrane (27). Photosensitized protein damage can produce important primary structural and functional changes through the photo-oxidation of five amino acid residues, namely cysteine, histidine, methionine, tryptophan, and tyrosine (16). Two of these residues readily oxidized by 1O_2 , cysteine and histidine, have been shown to play a critical role in regulation of the PT (7, 17).

Dithiol-disulfide interconversions at vicinal cysteines affect the pore open-closed transitions, a higher open probability being associated with the disulfide (7). The actual dithiol oxidant appears to be oxidized glutathione, which would mediate pore

opening by a variety of oxidants (23). Cysteine is oxidized to cystine by 1O_2 (16), and in the context of pore regulation by dithiols one could have predicted an increased probability of pore opening rather than the observed pore inactivation. These findings indicate that the inactivating effects of 1O_2 cannot be simply ascribed to cysteine oxidation leading to a dithiol-disulfide interconversion. The observed pore inactivation probably involves different SH groups or is the consequence of a conformational change brought about by photooxidation of other residues, which in turn prevents access of oxidized glutathione to the dithiol. This is supported by the finding that DIA, an SH group oxidant, is no longer effective at inducing pore opening in irradiated, HP-treated mitochondria; yet, pore opening under these conditions can still be observed by addition of the hydrophobic reagent PhAsO (Fig. 7), which reacts with vicinal dithiols to form a stable complex. Since pore opening by DIA and PhAsO is inhibited by *N*-ethylmaleimide and monobromobimane with the same apparent I_{50} (7, 28), we conclude that their site of action coincides, and we deduce that critical dithiols have not undergone oxidation as a result of irradiation.

The pore open-closed transitions are also regulated by reversible protonation of histidine residues from the matrix side of the membrane, with reversible pore closure at acidic matrix pH values that can be prevented by histidine carbethoxylation with DEPC (17). Free histidine residues can be photodegraded to several different compounds after cycloaddition of 1O_2 (29). Based on the inactivating effects of irradiation on the pore, one can speculate that histidine degradation following cleavage of the imidazole ring by 1O_2 may suppress the regulation of pore opening which depends on histidine protonation-deprotonation. Finally, our findings suggest that under normal conditions the role of histidines is in fact to allow rather than prevent opening of the pore, consistent with the optimum matrix pH for pore opening at 7.3 (17), *i.e.* a pH value at which histidines are largely deprotonated.

Among the identified mitochondrial targets for 1O_2 the AdNT stands out for its extreme sensitivity to photoinactivation (14), which can be traced to thiol group oxidation at sites critical for transport (30). Since the pore is affected by inhibitors of the AdNT, it has long been suggested that the translocase is involved, directly or indirectly, in pore formation or modulation (see Refs. 1, 2, and 25 for discussion). More direct evidence has recently come with the demonstrations (i) that the AdNT reconstituted in giant liposomes exhibits a striking Ca^{2+} -dependent, high-conductance channel activity with a marked voltage dependence (31) reminiscent of pore behavior both in isolated mitochondria (7) and in single channel measurements on mitoplasts (32); and (ii) that complexes enriched in hexokinase, porin, and the AdNT exhibit Ca^{2+} -dependent and cyclosporin A-sensitive high-conductance channel activity in planar lipid bilayers (33). While it is tempting to speculate that photoinactivation of the AdNT might be responsible for inactivation of the pore as well, we think that evidence remains circumstantial and that photoinactivation might well hit more than one critical target, including pore regulatory proteins like mitochondrial cyclophilin (34, 35).

Conclusions—In conclusion, the data presented in this paper support the view that photodynamic action leads to pore inactivation because of selective interference with critical histidine(s), rather than because of a generic pore denaturation, and suggest that the toxic consequences of the photodynamic effect in porphyrias and related disorders may involve an impairment of mitochondrial function also through inactivation of the pore, which is as sensitive to irradiation as is the AdNT. A thorough characterization of the complex consequences of histidine photooxidation on a variety of pore regulatory features

(like modulation by the membrane potential and by matrix pH) is currently under way in our laboratories.

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