Proton Selective Substate of the Mitochondrial Permeability Transition Pore: Regulation by the Redox State of the Electron Transport Chain†

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ABSTRACT: The permeability transition pore of rat liver mitochondria can be closed by chelating free Ca^{2+}, with respect to the passage of large molecules such as mannitol and sucrose. However, an apparent H^+-conducting substate remains open under these conditions, as indicated by the persistence of maximal O_2 consumption rates and by the failure to recover a membrane potential. Agents which favor a closed pore, such as cyclosporin A, ADP, Mg^{2+}, or bovine serum albumin, do not close the H^+-conducting substate, but it closes spontaneously when respiration becomes limited by the availability of O_2. Closure provoked by an O_2 limitation requires free Mg^{2+} in the sub-micromolar concentration range and becomes less efficient with increasing time spent in the presence of free Ca^{2+}. The H^+-conducting substate is apparently regulated by the redox status of the electron transport chain, with a reduced form favoring closure. A physical association (or equivalence) between the pore and one of the respiratory chain complexes is supported. These characteristics suggest that the transition is irreversible in vivo, if it involves a small fraction of total mitochondria, and would lead to their elimination and/or replacement by the cell. The implications of this proposal are considered, as they relate to a possible role for the transition in cellular apoptosis and the elimination of mitochondria containing mutated DNA.

The inner membrane of mitochondria contains a general diffusion pore which can open rapidly, causing a "permeability transition" that allows solutes of less than ~1.5 kDa to equilibrate their distribution (*1*, *2*). Since the identification of cyclosporin A (CsA)[1] as a potent inhibitor of the transition (*3*−*5*), the general diffusion pore has been held to be a highly regulated structure referred to as the permeability transition pore or PTP. PTP opening defeats the chemiosmotic mechanism of energy transduction and thus eliminates numerous functional activities of mitochondria. Accordingly, the occurrence and persistence of the transition in vivo, if involving a large fraction of mitochondria, would not be compatible with continued cell viability. This fact, together with the lack of solute selectivity, has contributed to uncertainty regarding the physiological function of the transition and of the PTP per se.

Current interest in potential functions of the PTP is focused on mechanisms of cell death. It is well established that PTP opening is a central event leading to necrotic cell death (*6*−*14*) and that the deleterious effects of an open PTP are not simply the result of uncoupling (*15*). Less certain, but supported by a growing body of evidence, is a role for the PTP in cell death occurring via apoptosis. In that case, it is release of cytochrome *c* from the intermembrane space (*16*−*19*), and/or of proapoptotic factors from the matrix space (*20*, *21*), which may be PTP-dependent and lead to cell death (but see refs *22* and *23*). The capacity of mitochondria to act as excitable structures (*24*) and to influence the characteristics of cytoplasmic Ca^{2+} waves (*25*, *26*) are additional areas of current interest where PTP involvement is postulated.

When contemplating potential roles of the PTP, it is important to consider how closure is regulated once opening has occurred and to take account of apparent PTP substates which would be more solute selective than the fully open form. In early studies, closure was brought about by lowering the prevailing Ca^{2+} concentration, and methods which are based on the transmembrane movement of relatively large molecules were utilized to monitor the process (*27*−*32*). These studies showed that closure occurs promptly upon Ca^{2+} chelation and that the closed state is subsequently maintained for extended periods. More recent studies have identified conditions which cause closure without reducing the Ca^{2+} concentration and have focused on the membrane permeability to H^+, as reflected by membrane potential ($\Delta\Psi$), as the parameter of interest. Those studies showed that many agents which inhibit PTP opening also promote closure, and that the open:closed probability is established through the collective action of multiple effectors acting at several regulatory sites (*33*, *34*).

With regard to PTP substates, the first indication that they exist came from comparing time courses for the transmembrane equilibration of small solutes following a rapid opening (*35*). Subsequent studies suggested that transient and

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[1] Abbreviations: CsA, cyclosporin A; PTP, permeability transition pore; P_i, inorganic phosphate; PEG, poly(ethylene glycol); $\Delta\Psi$, membrane potential; TEA^+, tetraethylammonium cation; TPP^+, tetraphenylphosphonium cation.

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partially open forms occur which retard the diffusion of mannitol/sucrose more effectively than metal cation and H^+ diffusion (*31*, *36*, *37*). Those apparent PTP substates may correspond to the short-lived, reduced conductance substates that have been observed using patch clamp techniques (*38*, *39*). The occurrence of substates is particularly apparent when pore opening occurs during an extended incubation in the presence of CsA and bovine serum albumin. Under those conditions, PTP-dependent movements of Mg^{2+} and mannitol/sucrose are separated by ~30 min, as though a metal cation-conducting substate arises initially and is slowly converted to a larger form that accepts mannitol/sucrose (*40*).

In this report, we demonstrate a substate which is long-lived in the absence of CsA, and which transmits H^+, but not mannitol/sucrose. The open or closed status of this form may depend on the redox state of the mitochondrial electron transport chain, with a reduced state promoting closure, and on the presence of Mg^{2+}. These findings are the first indication that the status of the respiratory chain may be a factor in PTP regulation. They also draw attention to potential physiological functions wherein occurrence of the transition in vivo, when involving a small fraction of total mitochondria, would be an irreversible event leading to removal of those mitochondria from the cell. Aspects of these findings have appeared in abstract form (*41*).

MATERIALS AND METHODS

Reagents. Poly(ethylene glycol) (PEG, 3.4 kDa) and tetraphenylphosphonium (TPP^+) chloride were obtained from Aldrich. Other reagents were obtained from commercial sources and were the best available grade. The mannitol/sucrose solutions used to prepare media were deionized and stored as previously described (*42*).

Preparation and Incubation of Mitochondria. Liver mitochondria were obtained from male Sprague-Dawley rats (~250 g) by a standard procedure (*43*). EGTA (0.5 mM) and bovine serum albumin (2 mg/mL) were present in the homogenizing medium, but were omitted from the washing medium which contained 230 mM mannitol, 70 mM sucrose, and 3 mM Hepes (Na^+) (pH 7.4). The final pellet was suspended at ~60 mg of protein/mL in washing media and maintained on ice. The protein concentration was determined by the Biuret reaction in the presence of 1% deoxycholate (Na^+).

Incubations comprised 25 mL and were conducted in a water-jacketed beaker which was open to the atmosphere (internal diameter = 2.8 cm, height = 5 cm). Moderate stirring was maintained throughout the experimental period. These conditions are specified because the diffusion of O_2 from the atmosphere into the media was a parameter of interest during the experiments presented. The temperature was 25 °C and the protein concentration was 0.5 mg/mL, unless otherwise noted. Media contained 10 mM succinate (Na^+), rotenone at 2.0 nmol/mg of protein, oligomycin at 1 μg/mL, 3 mM Hepes and 5 mM P_i (both Na^+) (pH 7.4), 2 μM TPP·Cl, and sufficient mannitol/sucrose (3:1 mole ratio) to give an osmotic strength of 300 mOsM. Further additions are described in the figure legends.

Other Methods. Swelling and contraction of mitochondria were monitored with a Brinkman probe colorimeter (PC 900) employing a 520 nm filter (*44*). The signal output from this instrument is in units of % T, and data are presented in that form. Changes in membrane potential ($\Delta\Psi$) were monitored by the accumulation or release of TPP^+, which was determined with an electrode. A TPP^+-impregnated membrane was prepared according to a literature procedure (*45*) and was used to convert a broken combination pH electrode to a combination electrode that is specific for TPP^+. During conversion, the internal reference solution was replaced with 10 mM TPP·Cl and a silicone sealant was used to fasten an area of membrane to the electrode barrel. The end of the barrel had been filed and polished to remove remnants of the original glass membrane. This electrode alleviates the need for an external reference and has maintained adequate operational characteristics for ~3 years. Washing the electrode surface with a dilute suspension of frozen and thawed mitochondria between incubations removes hydrophobic reagents from previous experiments and maintains the response time and sensitivity to TPP^+.

The medium O_2 concentration in the open vessel was monitored with a Clark-type electrode that was mounted in a plastic housing and connected to a Gilson Oxygraph. Because of the size of the incubation vessel, it was possible to measure this parameter simultaneously with swelling and membrane potential. Experiments were started by adding mitochondria, and no further additions were made until the distribution of TPP^+ reached a steady state.

RESULTS

During induction of the permeability transition by Ca^{2+} and P_i, a relatively constant relationship is maintained between the progression of swelling (mannitol/sucrose permeation) and the loss of $\Delta\Psi$ (H^+ permeation) when the overall rate is varied by changing the Ca^{2+} concentration (Figure 1). This behavior confirms earlier results which indicate that PTP substates are short-lived, at best, under conditions where PTP regulation is often investigated in vitro (*31*, *36*, *37*). Figure 1 also illustrates the transient recovery of $\Delta\Psi$ which is usually seen during the period between the initial depolarization caused by Ca^{2+} accumulation and a second depolarization caused by opening of the PTP. This transient repolarization is seen to a variable extent in the figures that follow because it reflects the severity of inducing conditions (Figure 1), and the characteristics of individual mitochondrial preparations.

Figure 2 shows an experiment similar to those in Figure 1, together with the change in medium O_2 concentration versus time and the effect of PEG on these parameters. The O_2 concentration trace does not represent a true rate of respiration because an open vessel having a substantial surface:volume ratio was employed (i.e., O_2 from the atmosphere can diffuse into the medium at a significant rate). Accordingly, and because a low protein concentration was employed (0.5 mg/mL), there is little evidence of the slow (state 4) respiration which is ongoing before Ca^{2+} is added to induce opening of the PTP. However, afterward, the medium O_2 concentration decreases at a specific rate of 108 ng of O atoms min^{-1} mg of $protein^{-1}$ (from Figure 2), and this value cannot be increased by the addition of uncoupler (data not shown). The apparent respiration rate is somewhat lower than a typical value for uncoupled rat liver mitochondria at 25 °C (~130−150 ng of O atoms min^{-1} mg of

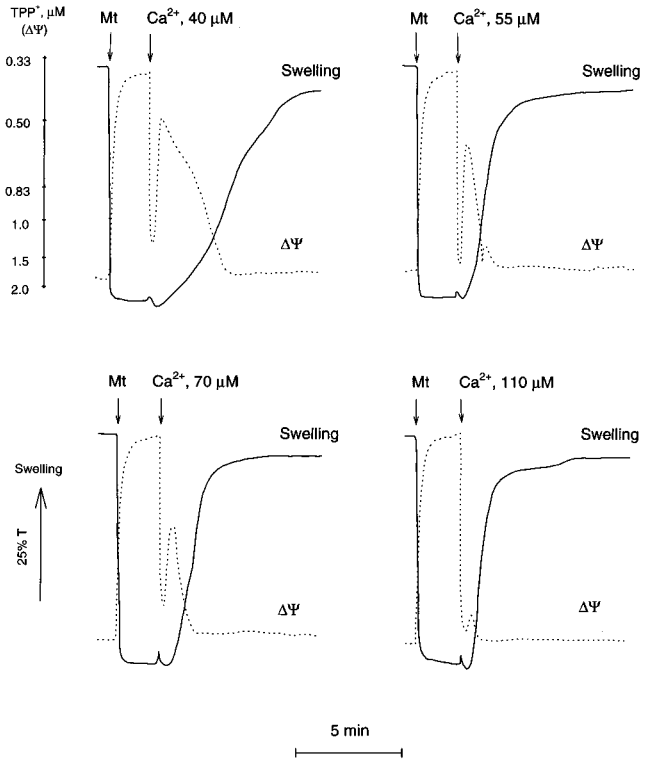

FIGURE 1: Time course of swelling and depolarization during the permeability transition. Mitochondria were incubated at a protein concentration of 0.5 mg/mL in a mannitol/sucrose-based medium containing 10 mM succinate, 5 mM P_i, and additional components described in Materials and Methods. Membrane potential ($\Delta\Psi$) (dotted lines) and swelling (solid lines) were monitored with a TPP^+ electrode and by optical methods, respectively, as also described in Materials and Methods. Conditions for the four panels were the same except that the level of $CaCl_2$ added was varied as shown.

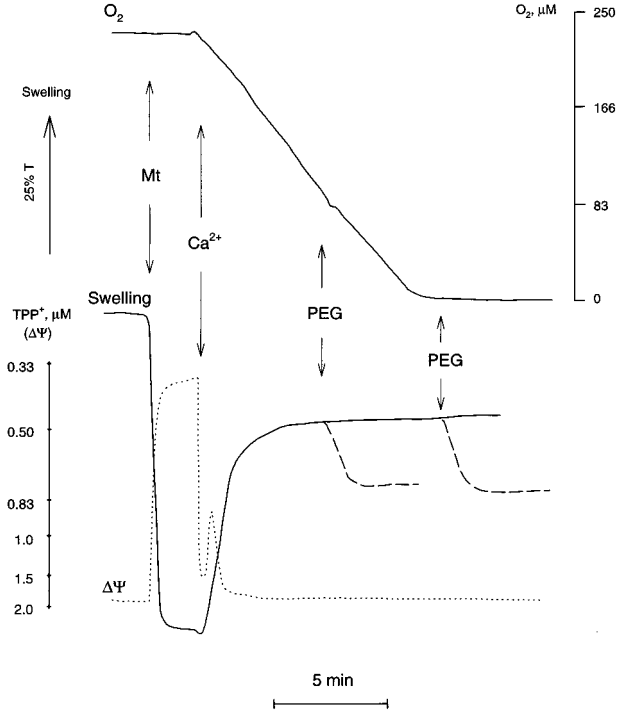

FIGURE 2: PEG-induced contraction of swollen mitochondria. Conditions were as described in the legend of Figure 1, except that the O_2 concentration was monitored together with $\Delta\Psi$ and swelling, as described in Materials and Methods. $CaCl_2$ was utilized at 140 nmol/mg of protein to induce opening of the PTP. Where indicated, 1.5 mM 3.4 kDa PEG was added and the contraction of mitochondria observed (dashed lines). Two experiments of this type are illustrated, in which PEG was added before or after the consumption of medium O_2.

protein^{-1}), again because O_2 can enter from the atmosphere and (perhaps) because matrix solute depletion via the PTP may reduce the capacity of the electron transport chain. Nevertheless, since the net rate of O_2 depletion is not increased by uncoupler, it can be concluded that the mitochondria are respiring maximally when the PTP is open. Under this condition, the electron transport chain should be highly oxidized (*46*, *47*).

During the period of rapid respiration, or after an (near) anaerobic condition has been obtained, the addition of 1.5 mM 3.4 kDa PEG causes a partial contraction of the swollen mitochondria, but has no effect on the rate of O_2 consumption or $\Delta\Psi$ (Figure 2). In these experiments, the initial swelling reflects an inward movement of mannitol/sucrose through the PTP, and a consequent accumulation of water, driven by the Donnan potential and the colloid osmotic pressure gradient derived from mitochondrial matrix proteins. PEG of 3.4 kDa is too large to enter the matrix space via the PTP (*30*, *48*) and thus reduces the colloid osmotic pressure differential when added to the incubation. When the PTP is open and swelling has occurred, the contraction which ensues represents the swelling mechanism operating in reverse and thus indicates that the PTP remains open with respect to mannitol/sucrose permeation (*49*−*51*). The magnitude of contraction seen in Figure 2 is relatively small because a low concentration of PEG was employed. However, it agrees well with the relationship between the concentration of 3.4 kDa PEG, when added initially, and the maximal extent of swelling which occurs upon opening the PTP (*48*). The extent of contraction can also be increased by increasing the concentration of PEG (data not shown). Thus, Figure 2 shows that permeation of mannitol/sucrose through the PTP is not affected much by the time interval since it was opened, or by the O_2 concentration in the medium.

When the Ca^{2+} concentration is reduced in media containing mitochondria with an open PTP, the structure closes with respect to mannitol/sucrose permeation (*28*, *30*−*32*, *52*). This is illustrated in Figure 3 by the absence of contraction when PEG is added following the addition of excess EGTA. Again, the effect of PEG is the same when the O_2 concentration is high or low. More notable is the continuing high rate of O_2 consumption and the absence of $\Delta\Psi$ which are still observed in the presence of EGTA (Figure 3). Taken together with the absence of a PEG-induced contraction, these findings indicate that when the PTP closes with respect to mannitol/sucrose permeation, the inner membrane remains highly permeable to H^+.

Six to seven minutes following addition of EGTA, the inner membrane repolarizes spontaneously (Figure 3). In what seems initially to be a paradox, the time at which this occurs coincides, approximately, with the apparent exhaustion of O_2 in the medium. However, as emphasized above, O_2 is able to diffuse into the medium because the apparatus which contained these incubations was open to the atmosphere. Accordingly, some respiration remains possible at and beyond the time when repolarization occurs. To confirm that the repolarization is real and dependent on continuing

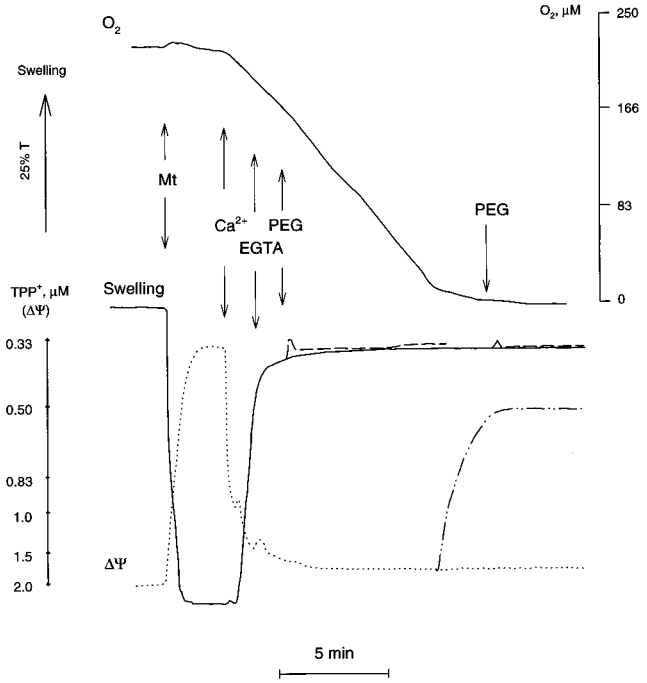

FIGURE 3: H^+ permeability following closure of the PTP. Conditions were as described in the legend of Figure 2, except that EGTA (0.50 mM) was added after Ca^{2+} addition and opening of the PTP, as illustrated in the figure. Dashed lines associated with the swelling trace show that PEG addition does not cause contraction of mitochondria when EGTA is present. The dashed and dotted line associated with the $\Delta\Psi$ trace shows the repolarization which occurs spontaneously when EGTA is present and the medium O_2 concentration approaches zero.

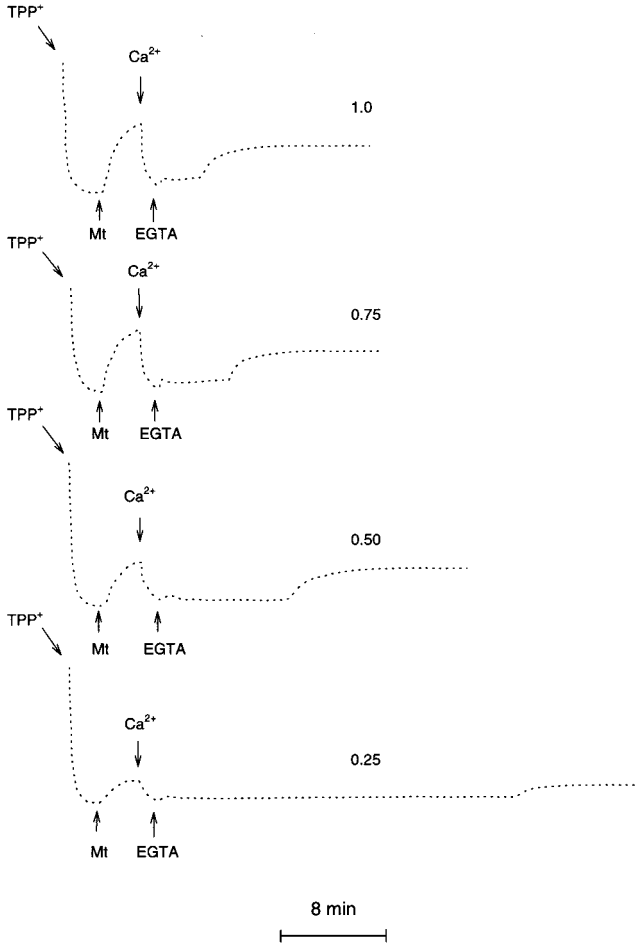

FIGURE 4: Effect of protein concentration on repolarization. All traces show $\Delta\Psi$ (TPP^+ concentration) determined as described in Materials and Methods and the legend of Figure 1. The concentration of the mitochondrial protein was 1.0, 0.75, 0.50, or 0.25 mg/mL, as indicated by the number associated with each trace (right side). Where indicated, $CaCl_2$ and EGTA were added at 300 nmol/mg of protein and 0.5 mM, respectively.

respiration, we determined that uncoupler, antimycin A, CN^-, or the presence of a N_2 atmosphere above the incubation vessel prevents the phenomenon when added before it occurs, reverses it when added after it occurs, and that the same behavior is seen when mitochondria are oxidizing ascorbate/TMPD rather than succinate (data not shown).

We also determined the effect of protein concentration on the time interval required to obtain repolarization following addition of EGTA. As seen in Figure 4, this interval increases as the protein concentration decreases. O_2 concentration data (not shown) demonstrated that the time of repolarization coincides approximately with the time required to obtain a near-anaerobic state in all cases (i.e., coincides with the point where respiration becomes limited by O_2 diffusion into the medium). Furthermore, at a given protein concentration, the use of media supersaturated with O_2 lengthens the time proportionally (data not shown). Thus, the correspondence which was seen in Figure 3 is not a coincidence, but indicates that an O_2 limitation is required to obtain repolarization. When respiration of uncoupled mitochondria becomes limited by O_2 availability, the electron transport chain is shifted from primarily oxidized to primarily reduced (*46*, *47*).

Several agents which antagonize opening of the PTP, or promote its closure, were tested to determine their effect on recovery of $\Delta\Psi$ during the period between addition of EGTA and the onset of an O_2 limitation. CsA, ADP, and Mg^{2+} were ineffective when used alone or in combinations. Bovine serum albumin, in excess of free fatty acids that arise under these conditions (*40*), was also ineffective (data not shown).

However, when EDTA rather than EGTA was used to chelate Ca^{2+}, $\Delta\Psi$ was not recovered when the medium O_2 concentration reached a low value, as in the other experiments (Figure 5, trace 1). The addition of Mg^{2+} together with EDTA restored the behavior seen when EGTA is used to chelate Ca^{2+} (Figure 5, trace 2), or allowed a recovery of $\Delta\Psi$ when added after the O_2 limitation had been obtained (Figure 5, trace 3). This apparent Mg^{2+} requirement for fully reversing the permeability transition is noteworthy because the PTP was closed to mannitol/sucrose on EDTA addition without exogenous Mg^{2+} (Figure 5). In addition, and as further discussed below, the site where Mg^{2+} acts to promote full closure is apparently of high affinity because the exogenous cation is effective even when EDTA is present in excess of total Ca^{2+} and Mg^{2+}.

Finally, the time of chelator addition is another factor which influences the recovery of $\Delta\Psi$ as O_2 availability begins to limit the rate of respiration. If EGTA is added within the first minute after swelling reaches completion, recovery is prompt when it ultimately occurs. However, as the time preceding chelator addition is increased, the subsequent recovery of $\Delta\Psi$ becomes more sluggish (Figure 6). This is in contrast to the recovery of a permeability barrier for mannitol/sucrose which is prompt and complete,

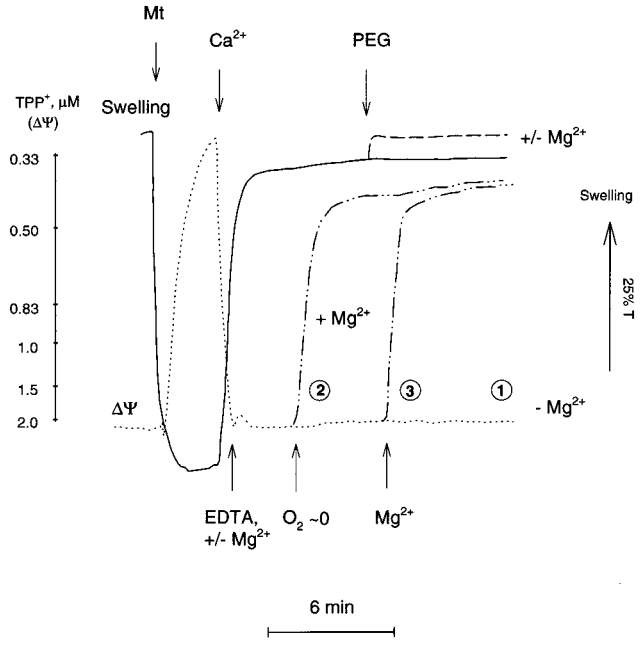

FIGURE 5: Effect of Mg^{2+} concentration on repolarization. Conditions were as described in the legend of Figure 2, except that EDTA (0.40 mM) rather than EGTA was added after opening the PTP, and $MgCl_2$ (100 μM) was also added in some cases, as illustrated in the figure. Dashed lines associated with the swelling trace show that PEG addition does not cause contraction of mitochondria when EDTA is present, regardless of whether or not Mg^{2+} has been added. The dashed and dotted lines reflecting $\Delta\Psi$ show how exogenous Mg^{2+} effects repolarization when EDTA is present: trace 1, Mg^{2+} not added; trace 2, Mg^{2+} added at the same time as EDTA; and trace 3, Mg^{2+} added following EDTA, after an O_2 limitation had been attained, as illustrated in the figure. The medium O_2 concentration was monitored, as in other figures, so that the time when O_2 availability begins to limit respiration could be ascertained. These traces are not shown to maintain clarity in the figure.

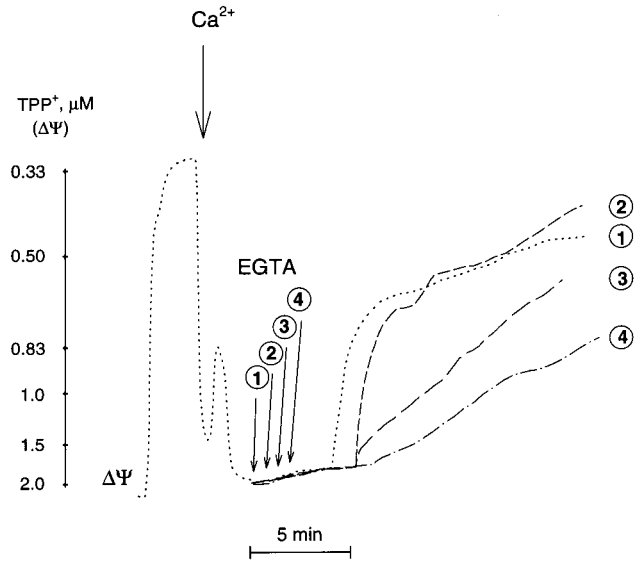

FIGURE 6: Time dependence of repolarization. Conditions were like those described in the legend of Figure 3 except that the time of EGTA addition was varied as shown. Numbers associated with the individual arrows and traces identify which time of addition led to which time course of repolarization.

as judged by the absence of PEG-induced contraction, even when EGTA addition is delayed for several minutes (data not shown).

DISCUSSION

The inner membrane structure which maintains an uncoupled condition after large forms of the PTP are closed (Figure 3) is manifest in the absence of free Ca^{2+} and is not closed by CsA, ADP, or bovine serum albumin which are well-known inhibitors of large forms. Accordingly, it is possible that the more selective structure is not derived from the PTP, or that uncoupling reflects a rapid futile cycle of monovalent cation transport involving more selective activities. The latter possibility seems improbable because substituting K^+ or TEA^+ for Na^+, as the exogenous medium cation, has little effect on the maintenance of uncoupling following Ca^{2+} chelation, on the recovery of $\Delta\Psi$ as O_2 limitation is attained, or on the influence of Mg^{2+} on these parameters (data not shown). Thus, both the uniport and antiport components of the putative futile cycles would necessarily be nonselective, or multiple selective transporters with high activities would be present. Neither possibility seems probable, based upon the known monovalent cation transport activities of liver mitochondria (*53*, *54*). However, there is no analogous objection to the occurrence of a H^+ selective PTP substate or its postulated role in the maintenance of uncoupling.

The possibility that the H^+ selective substate is, in fact, derived from non-PTP structures will remain until the composition and structure of the PTP per se have been determined. Along this line, it is important to note that the often stated PTP characteristic of accepting solutes with a molecular mass of $\leq$1.5 kDa is derived from an early study in which PEGs of several molecular masses were used to estimate the parameter (*30*). Recent and more extensive studies suggest that the PTP is of different sizes in individual mitochondria and that the distribution of sizes varies with the inducing conditions (*48*, *55*). Thus, the present data may be seen as an addition to a growing body of evidence which does not support the PTP being a single molecular entity.

The oxidation-reduction status of mitochondrial respiratory chain complexes is not easily determined at the protein concentrations employed here, and timing/activity factors inherent in these experiments make it difficult to work at higher concentrations. Nevertheless, it is clear from Figure 2, and from a great deal of earlier data, that PTP opening fully uncouples mitochondria and that the respiratory chain is relatively oxidized under such conditions. Similarly, it seems clear that the chain will become more reduced as a decreasing medium O_2 concentration begins to limit the rate of respiration. Accordingly, the present data suggest that a relatively reduced state of the electron transport chain is required to close the PTP substate which conducts H^+ but not mannitol/sucrose. Alternatively, the substate could be controlled through a distinct site which binds O_2 with an affinity similar to that of cytochrome oxidase, with closure occurring when the site is not occupied. No clear distinction between these possibilities is possible on the basis of the present data; however, it is still useful to explore their implications, as they relate to regulation of the PTP and its potential physiological function.

With respect to regulation, it is important to note that the influence of O_2 concentration is manifest after the pore has previously been opened fully, allowing the release of matrix space cofactors and coenzymes (*1*, *2*). If it is assumed that

it is redox state rather than an isolated O_2 binding site which is involved, this fact suggests a physical association, or an equivalence, between the PTP and one or more of the respiratory chain complexes. This is because, with the prior depletion of redox-coupled coenzymes, it is difficult to envision how a regulatory influence of redox status could be transmitted to the PTP in the absence of a physical interaction. It will be interesting to determine which of the respiratory chain complexes is involved, particularly in view of the Mg^{2+} requirement for closing the H^+-conducting form (Figure 5). Calculations using the program Bound and Determined (*56*) show that the free Mg^{2+} concentration established to promote closure under the conditions of Figure 5 was 0.4 μM (compared to ~9 nM for free Ca^{2+}). Accordingly, the site at which Mg^{2+} acts is of high affinity and probably a component of the structure which is transmitting H^+. Subunits I and II of the cytochrome oxidase complex, acting in concert, bind one Mg^{2+} with high affinity (*57*−*59*). This site is located near the heme a_3−Cu_B center where structures conducting H^+ and H_2O through the complex are thought to converge (*60*). Accordingly, Mg^{2+} may be acting at this site, a possibility which is further consistent with the redox active nature of the complex and its regulation through adenine nucleotide binding (*60*, *61*). Thus, the cytochrome oxidase complex may be subject to a Mg^{2+}-related and reversible structural and/or conformational conversion under conditions which promote the permeability transition, which converts a vectorial H^+-transporting domain to a H^+-conducting channel.

In considering how the present results bear upon potential physiological functions of the PTP we first note the scarcity of data indicating that the phenomenon occurs in normal cells (reviewed in ref *40*). This might indicate that the transition simply does not occur unless cells are proceeding toward death via apoptosis or necrosis. However, it is also possible that, in vivo, only a few mitochondria are in the PTP open condition at any one time and that these have so far escaped detection. If the latter is assumed, the present data suggest that occurrence of the transition in vivo would be an irreversible event. This is because with a few mitochondria uncoupled, while the majority remained coupled, it is not likely that the local O_2 concentration would become low enough to limit respiration. Thus, a reduced respiratory chain would not be recovered and the proton selective substate would remain open.

Several factors suggest that persistence of an open PTP would lead to the loss of those particular mitochondria from the cell. For example, Skulachev (*74*) has pointed out that protein import would be arrested because it is driven by $\Delta\Psi$ and that ultimately this would lead to degradation. Beyond that, mitochondria contain nucleases, proteases, and phospholipases which are poorly understood from functional and regulatory perspectives (*62*−*64*). Presumably, these endow the organelle with some, but as of yet an unspecified, capacity for autolysis, which could lead to direct removal of mitochondria that have undergone the transition. Supporting this possibility are the known activation of phospholipases which accompanies the transition (*1*) and the actions of phospholipase and (potentially) protease reaction products which favor an open PTP (*40*, *48*). An accumulation of these products, coupled with a generalized autolysis following the transition, may explain why it becomes more difficult to close the H^+-conducting substate as Ca^{2+} chelation is delayed (Figure 6). Thus, known actions of hydrolytic products on the PTP open:closed probability can be viewed as helping to ensure that opening remains irreversible as mitochondrial degradation proceeds.

Mitochondrial DNA mutations are associated with numerous late onset diseases (*65*) and may play an underlying role in aging (*66*, *67*). The accelerating rate at which these mutations accumulate in aging human skeletal muscle (*68*), for example, has been taken to indicate that the individual mitochondria involved are replicated in preference to normal mitochondria (*69*). However, it is also possible that mitochondria which harbor mutations are subject to a negative selection which is more effective in young compared to old individuals. The mitochondrial permeability transition, when viewed as an irreversible event leading to loss of the specific mitochondrion involved, has characteristics expected of a mechanism giving rise to negative selection. This is because nearly all mutations in the mitochondrial genome are expected (perhaps after a threshold has been obtained) to diminish proton motive force and possibly to provoke O_2 radical production. These factors and their consequences promote opening of the PTP (*70*−*72*) and would then lead to elimination of the mutated forms.

Others have noted that the permeability transition may lead to removal of damaged or unwanted mitochondria (*73*−*75*), an idea which now has an experimental basis. When viewed in this way, PTP opening and the subsequent loss of a mitochondrion become analogous in purpose to cellular apoptosis which removes damaged or unwanted cells (*75*). Since cytochrome *c* and other factors which promote cellular apoptosis are released from mitochondria following the transition, a continuum may exist between this "mitochondrial apoptosis", cellular apoptosis, and cell death via necrosis. That is to say, conditions giving rise to a small number of poorly functioning mitochondria would lead only to their removal and/or replacement because relatively small amounts of the factors would be released into the cytoplasm. More severe conditions, affecting larger fractions of mitochondria, would provoke cellular apoptosis because a threshold was crossed with respect to the level and persistence of these factors. In the extreme, where most mitochondria were involved, the role of pore opening in necrotic cell death would be manifest. Further testing of these interpretations is in progress.

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