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Correlation between ATP synthesis, membrane potential and oxidation rate in potato mitochondria

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Using a tetraphenylphosphonium-sensitive electrode, the same relationships between membrane potentials and oxidation rates were observed in potato-mitochondria oxidizing succinate under phosphorylating or nonphosphorylating conditions. This relationship was not verified when the oxidation rates were limiting. Under all conditions, membrane depolarization in phosphorylating states, when the rates of oxidation or phosphorylation were inhibited, behaved as if oxidation and phosphorylation were coupled by the protonmotive force. Furthermore, the availability of protons for ATP synthesis appeared the same as for protonophores or the mitochondrial H^+/K^+ antiporter. These results are not consistent with a direct interaction between the ATP-synthase and the respiratory chain in plant mitochondria.

Introduction

According to the chemiosmotic hypothesis, oxidative phosphorylation is mediated by a proton gradient created by substrate oxidation and used by the ATP synthase to synthesize ATP [1]. Although the proton gradient is still accepted as such an intermediate, some authors, by measuring the correlation between $\Delta\tilde{\mu}H^+$, the electrochemical proton gradient, and the kinetic parameters of mitochondrial respiration and ATP synthesis, have found results more consistent with a localized proton circuit [2,3] or a direct interaction between the respiratory chain and the ATP synthase [4,5]. Indeed, discrepancies have appeared between these parameters, ATP synthesis and the oxidation rates being greatly modified with only slight changes in $\Delta\tilde{\mu}H^+$ [3]. Furthermore, the rates of oxidation under phosphorylating or nonphosphorylating conditions have been found to be correlated differently to the extents of $\Delta\tilde{\mu}H^+$ (see Ref. 6).

Previous investigations have shown that in plant mitochondria the membrane potential ($\Delta\psi$) is very high (220–240 mV) [3,7] compared to that in animal mitochondria (150–180 mV) [8–10]. Furthermore, very little effect of nigericin, which diminishes ΔpH by exchanging H^+ and K^+, was observed with plant mitochondria [3,7]. The presence of an active H^+/K^+ exchanger [11] could account for the low ΔpH observed in plant mitochondria. As a consequence, $\Delta\psi$, being not affected by such electroneutral exchanges and appearing very important in plant mitochondria, could be a suitable criterion of ions fluxes (e.g., proton extrusion and proton influx) across the inner mitochondrial membrane. In the present study the relationships between $\Delta\psi$, oxidation rates

Abbreviations: CCCP, carbonyl cyanide-*m*-chlorophenyl hydrazone; TPB^-, tetraphenylboron; TPP^+, tetraphenylphosphonium; $\Delta\tilde{\mu}H^+$, electrochemical proton gradient; $\Delta\psi$, bulk-phase potential.

and phosphorylation rates have been investigated using potato mitochondria. Two main conclusions emerged from this study: a strong correlation exists between $\Delta\psi$ and the oxidation rates in phosphorylating as well as in nonphosphorylating states; proton influx occurs in the same way through either the ATP-synthase or protonophores or the H^+/K^+ antiporter.

Materials and Methods

Preparation of mitochondria

Extraction and purification of mitochondria from potato tubers (*Solanum tuberosum* L.) were carried out as previously described [12]. Purification was performed on a 22.5% (v/v) self-generating Percoll gradient. Purified mitochondria were collected in a medium containing 0.3 M mannitol/1 mM EDTA acid/1% (w/v) bovine serum albumin (pH 7.2). Mitochondrial protein was determined by nesslerization.

Measurements of respiratory activities

Oxygen consumption, membrane potential and oxidative phosphorylation were determined simultaneously in a single experiment. Measurements were carried out at 25°C in a glass vessel (final volume, 2 ml) in a medium containing 0.3 M mannitol/5 mM $MgCl_2$/30 mM KCl/1% (w/v) bovine serum albumin/10 mM potassium phosphate buffer (pH 7.2). About 0.8–1.2 mg protein was added per assay. Oxygen uptake was measured polarographically with a Clark-type electrode (Hansatech Ltd, King's Lynn, U.K.) and membrane potential was measured with a TPP^+-sensitive electrode [8]. A minielectrode was constructed with a poly(vinyl chloride) tube sealed with a poly(vinyl chloride)-based membrane containing TPB^- as a cation exchanger and filled with a 10 mM TPP^+ solution. The reference electrode was an Ag|AgCl-saturated electrode (Tacussel, model MI 402). The signals were simultaneously fed to a dual-trace recorder (Servotrace, model PED X 100). The concentration of TPP^+ in the assay medium was usually 10 μM and no alteration in the oxidative properties of purified potato mitochondria was observed under these conditions. The calibration of the minielectrode was obtained with several additions of TPP^+ before addition of mitochondria, each addition doubling the concentration of TPP^+ in the medium up to 10 μM. Each doubling of the TPP^+ concentration gave the same variation of electrode potential: 17.8 mV [8].

Mitochondria were added after calibration of the electrode and were incubated in the presence of ATP (100 μM) to obtain both a maximal rate of oxidation [13] and a maximal TPP^+ uptake after addition of the respiratory substrate. The addition of 5 mM succinate caused a rapid TPP^+ uptake due to the formation of a membrane potential (negative inside) across the inner mitochondrial membrane. The subsequent addition of ADP (in order to obtain a steady-state-3 membrane potential) was linked to a TPP^+ efflux from the mitochondria and a rapid uptake of TPP^+ occurred during the establishment of the respiratory control. The addition of a saturating concentration of valinomycin, a K^+ specific ionophore, caused a total collapse of the membrane potential and the value of the electrode potential after this addition was used as a reference for all $\Delta\psi$ determinations [7]. An intramitochondrial volume of 1 μl/mg protein [3] was assumed for the calculation of $\Delta\psi$ by using the equation of Kamo et al. [8].

Modification of oxidative-phosphorylation rates

The experiments in which the membrane potential was progressively collapsed (valinomycin, valinomycin + nigericin, CCCP) were performed by adding increasing concentrations of the ionophore or protonophore during the course of the experiment (valinomycin: 0–20 ng per mg protein; CCCP: 0–1 μM). Where indicated 0.1 μg nigericin per mg protein was present at the beginning of the assay. TPP^+ concentrations (giving $\Delta\psi$) and oxidation rates were measured simultaneously. For the experiments in which the parameters of oxidative phosphorylation are modified, each point represents a single experiment. $\Delta\psi$ and the oxidation rate were measured during the first phosphorylation cycle.

Modifications of oxidation rates were performed using malonate, a competitive inhibitor of succinate dehydrogenase. Malonate (0–1.25 mM) was added before the addition of ATP and succinate.

Modifications of phosphorylation rates were

performed using two different methods. The availability of ADP for the ATP synthase was limited by the use of carboxyatractyloside, a specific inhibitor of the adenine nucleotide translocator, or the rate of ADP supply was limited with an hexokinase-glucose system. Atractyloside (0–0.2 μM) was added before succinate. Hexokinase (0–17 nkat) was added to the reaction medium before addition of succinate. In the presence of limiting concentrations of hexokinase, the state-4–state-3 transitions were obtained by adding saturating concentration of glucose (3 mM).

Results

Experimental trace with the TPP^+-sensitive electrode

A typical experimental trace is shown on Fig. 1. After calibration of the electrode with increasing concentrations of TPP^+ (2.5 + 2.5 + 5 = 10 μM), purified mitochondria were added to the assay. A little change in the TPP^+ concentration was observed, due partly to the dilution of the TPP^+ concentration by the addition of mitochondria and partly to a non energy-dependent TPP^+ uptake by the mitochondrial suspension. Addition of 100 μM ATP did not affect the response of the electrode. The addition of 5 mM succinate caused the start of the O_2 uptake and a decrease in TPP^+ concentration in the medium, indicating a TPP^+ uptake by the mitochondria and the generation of a transmembrane potential. When mitochondria were incubated for a few seconds in the presence of ATP before addition of succinate, the TPP^+ uptake was maximal (as in further state 4). A membrane potential of 220–230 mV was usually measured with purified potato mitochondria. The addition of ADP resulted in a concomitant increase in oxidation rate and a release of TPP^+ from the mitochondria, the value of the membrane potential in state 3 under the conditions of the Fig. 1 was about 190 mV. The decrease in oxidation rate between state 3 and state 4 was associated with an increase in membrane potential which returned to the value obtained after the addition of succinate. The addition of an uncoupler (CCCP or valinomycin in the presence of K^+) caused a total collapse of the membrane potential. The TPP^+ concentration in the medium returned to the value obtained after the addition of mitochondria, indicating that usually no TPP^+ binding occurred during oxidation or phosphorylation in purified potato mitochondria.

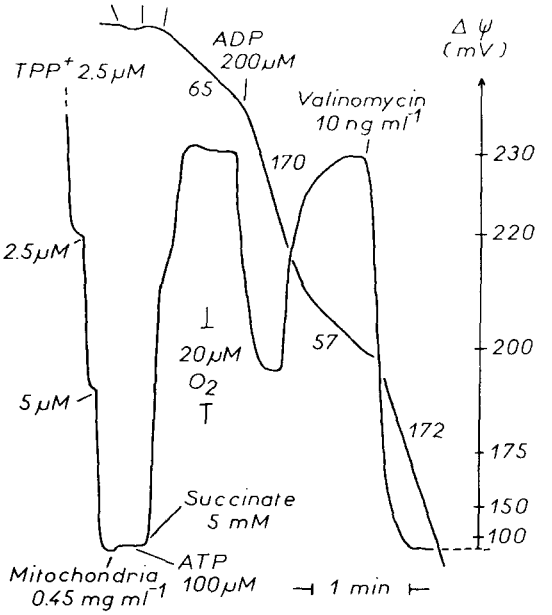

Fig. 1. Simultaneous recording of oxidation rates and changes in TPP^+ concentration. The assay was preceded by a calibration of the TPP^+-sensitive electrode with additions doubling the TPP^+ concentration in the medium to a final concentration of 10 μM. Assays were carried out at 25°C in 2 ml of the assay medium (see Materials and Methods). Where indicated purified mitochondria (about 30 mg protein/ml) were added to give a final concentration of about 0.5 mg protein/ml. ATP (100 μM) was added before addition of succinate (5 mM). A sufficient addition of ADP (200 μM) gave a steady-state-3 rate of O_2 uptake. The logarithmic span of the calculated [8] membrane potential is indicated, giving an idea of the limitation of the method: potentials below 100 mV are not measurable by this method. The rates of O_2 uptake are indicated on the polarographic trace.

Uncoupling conditions

In Fig. 2, membrane potentials were plotted against oxidation rates when the ionophore concentrations were progressively increased in state 4. The addition of limiting amounts of ionophores caused a concomitant collapse of $\Delta\psi$ and an increase in the oxidation rates. The curves obtained with increasing valinomycin concentrations in the presence or in the absence of nigericin are pre-

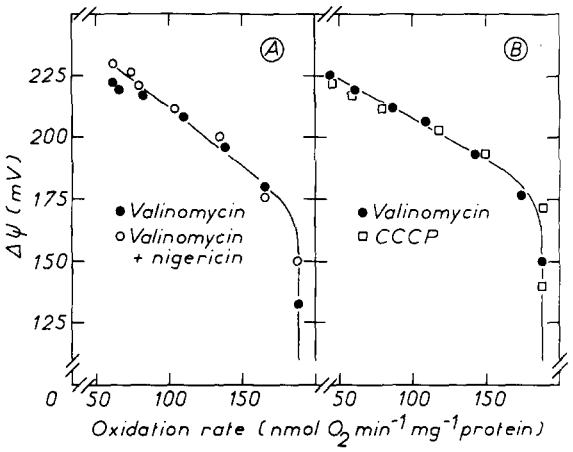

Fig. 2. Correlation between $\Delta\psi$ and oxidation rate in the presence of increasing concentrations of ionophore. These curves were obtained by simultaneously measuring $\Delta\psi$ and the oxidation rates. (A) The membrane potential was progressively collapsed with valinomycin (0–20 ng per mg protein) in the absence (●) or in the presence (○) of nigericin (0.1 μg per mg protein) and 30 mM K^+. (B) The membrane potential was collapsed with valinomycin (0–20 ng per mg protein, ●) in the presence of 30 mM KCl or with CCCP (0–1 μM, □).

sented in Fig. 2A. A linear relationship was obtained until the maximal oxidation rate was reached. Then no correlation could be found between $\Delta\psi$ and the oxidation rate (similar curves have been obtained between $\Delta\tilde{\mu}H^+$ and the oxidation rate by Azzone et al. [14] for animal mitochondria). The presence of nigericin did not affect the curve significantly, a slight effect could be observed only for the lowest valinomycin concentrations. Under these conditions $\Delta\psi$ was enhanced by 5–10 mV in the presence of nigericin, which diminished ΔpH by exchanging H^+ and K^+ (see also Refs. 3 and 7).

In Fig. 2B the effect of a protonophore (CCCP) is compared to the effect of valinomycin. The same relationship between $\Delta\psi$ and the oxidation rate was obtained in these two instances. The effect of valinomycin on this relationship appeared equivalent to an increase in the proton influx. It can be seen from Fig. 2 that, whatever the artificial means used to increase the membrane conductance to ions, the same relationship was obtained between $\Delta\psi$ and the oxidation rate.

Valinomycin was shown to be a specific K^+ ionophore (see Ref. 15) but its effect is very close to that of a protonophore (Fig. 2). The experiment presented in Fig. 3 was carried out in order to specify this effect of valinomycin on plant mitochondria. In the absence of valinomycin a slow swelling was observed in 0.1 M KCl, the addition of a limiting amount of valinomycin (10 ng per mg protein) induced a rapid swelling. The inner mitochondrial membrane appeared slightly permeable to K^+ in nonenergized plant mitochondria (Fig. 3A) and freely permeable to Cl^-, which appeared to enter plant mitochondria by passive diffusion [16]. The energization of mitochondria by adding succinate (Fig. 3B) did not enhance the swelling in the absence of valinomycin but a rapid swelling occurred in its presence. This swelling was followed by a shrinking corresponding to an efflux of K^+ from the mitochondria and a steady state was reached. This shrinking was sensitive to uncouplers, and a slow influx of K^+ occurred after addition of CCCP (Fig. 3B). The presence of CCCP before the addition of valinomycin caused a very fast swelling and no shrinking occurred (Fig. 3C). In the presence of substrate and of limiting amounts of valinomycin (which do not totally collapse $\Delta\psi$), a CCCP-sensitive efflux of K^+ occurred. A saturating concentration of valinomycin (20 ng per mg

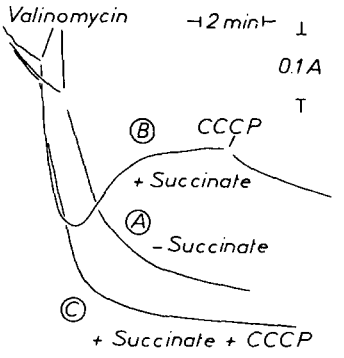

Fig. 3. Mitochondrial swelling of purified potato mitochondria in KCl medium. The same concentrations of mitochondria as for respiratory activities were used in these experiments (about 1 mg protein in 2 ml of KCl medium). Where indicated, 10 ng valinomycin per mg protein and 1 μM CCCP was added. (A) No substrate added; (B) mitochondria were under state 4 (5 mM succinate); (C) substrate (5 mM succinate) was added with CCCP before addition of valinomycin. The swelling of mitochondria was estimated in KCl medium (0.1 M) by measuring the absorption at 520 nm.

protein) suppressed this efflux (results not shown). This K^+ efflux appeared dependent on membrane energization, since no K^+ efflux could be seen when $\Delta\tilde{\mu}H^+ = 0$ (no substrate, saturating valinomycin or CCCP). In consequence, the uncoupling effect of valinomycin in plant mitochondria could be attributed to a $\Delta\psi$-dependent K^+ cycling [17] mediated by the ionophore and a mitochondrial H^+/K^+ antiporter [11,18]. In energized potato mitochondria the effect of valinomycin could thus be equivalent to a proton reentry linked to the K^+ cycling.

Phosphorylating conditions

Phosphorylation efficiency was measured with potato mitochondria progressively uncoupled by valinomycin. As already seen in Fig. 2, the presence of valinomycin decreased $\Delta\psi$ and simultaneously increased the state-4 rate. Under these conditions the state-3 rate was not varied and the respiratory control ratio was progressively decreased. With limiting concentrations of valinomycin ($\Delta\psi$ decreased from 220 mV to 150 mV), the ADP/O ratio decreased from 1.5 in the absence of valinomycin to about 0.3 in the presence of 15 ng valinomycin per mg protein (Fig. 4).

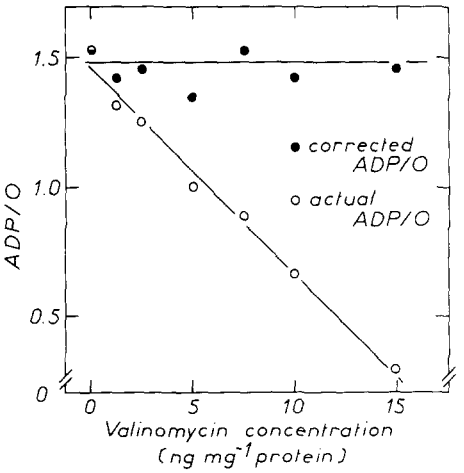

Fig. 4. Phosphorylation in presence of increasing concentrations of valinomycin. The conditions of measure are described in Materials and Methods. Each actual ADP/O value (○) was measured on a single experiment. ADP/O was calculated from the polarographic traces [24] using the total time of state-4 → state-3 → state-4 transition measured on the TPP^+ electrode trace. Corrected ADP/O values (●) were calculated as described in the text.

By subtracting the O_2 consumption released by the addition of valinomycin from the total O_2 consumed during the phosphorylation time a 'corrected ADP/O ratio' could be calculated and plotted against the valinomycin concentration (Fig. 4). It can be seen that this corrected ADP/O remained at a quite constant value for valinomycin concentrations increasing from 0 to 15 ng per mg protein. Such a result verified the initial hypothesis of the correction procedure, namely that the extra-oxygen consumption due to the presence of valinomycin was the same during state 4 as in state 3. In terms of H^+ fluxes, these results showed that the uncoupling effect of valinomycin was linked to a nonphosphorylating proton reentry and that this proton flux continued at the same rate under phosphorylating conditions. This proton flux, linked to the K^+ cycling, appeared to be limited only by the valinomycin concentration and was not available for ATP synthesis, suggesting that no preferential proton circuit exists between the ATP synthase and the respiratory chain.

The simultaneous measurement of $\Delta\psi$ and the oxidation rate in state 3 was performed when phosphorylation was limited. Fig. 5A presents the results obtained with the two methods used (see Materials and Methods). With the hexokinaseglucose system, the concentration of hexokinase was the limiting factor and phosphorylation was started by the addition of glucose. Carboxyatractyloside (a specific inhibitor of the adenine nucleotide translocator) inhibited the rate of phosphorylation by limiting the availability of ADP to the ATP synthase.

With the same mitochondrial preparation, the two methods used to control the rate of phosphorylation (and consequently the state-3 rate of oxidation) provided plots which were identical (Fig. 5A). The dotted line represents the curve obtained in the same experiment by using increasing valinomycin concentrations (cf. Fig. 2A) instead of inhibiting the phosphorylation rates. Such a comparison indicates that identical relationships between $\Delta\psi$ and the oxidation rate were obtained when the proton influx was carried through the ATP synthase to synthesize ATP or directly through inner mitochondrial membrane under the effect of valinomycin and the H^+/K^+ antiporter. The first point of the hexokinaseglucose curve was

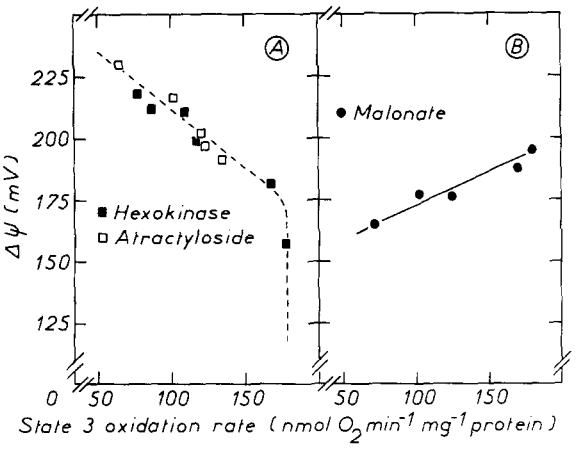

Fig. 5. Relationship between $\Delta\psi$ and oxidation rates when the phosphorylation rate or the oxidation rate are limited; see Materials and Methods for experimental conditions. (A) The phosphorylation rate was limited by the hexokinaseglucose system or by carboxyatractyloside: each point (■) represents a single trace. The conditions were: 200 μM ATP, hexokinase (0–17 nkat). Phosphorylating conditions are induced by the addition of 3 mM glucose. These points (□) were obtained when phosphorylation of 200 μM ADP was performed in the presence of increasing concentrations of carboxyatractyloside (0–0.2 μM). The dotted line was obtained in the same experiment by increasing the valinomycin concentration (see Fig. 2). (B) (●), the oxidation of succinate was inhibited by malonate (0–1.25 mM).

obtained without adding hexokinase, because a low endogenous hexokinase activity is present in purified potato mitochondria as in other plant mitochondria [19]. In these experiments, in which phosphorylation was limited, the phosphorylation efficiency (ADP/O ratio) could only be calculated in the case of the inhibition by carboxyatractyloside. Under these conditions, the ADP/O ratio decreased with increasing carboxyatractyloside concentrations and varied from 1.5 (no inhibitor) to 0.4 when 0.2 μM carboxyatractyloside was present.

In Fig. 5B malonate was used to control the respiratory rate; $\Delta\psi$ and respiratory rates were measured in the presence of ADP at increasing concentrations of malonate. The picture is very different from that in Fig. 5A. When the malonate concentration was increased from 0 to 1.25 mM, the decrease of membrane potential in state 3 was proportional to the decrease in the oxidation rate. Under the conditions of this assay, only the rate of oxidation in state 3 was limited (approx. 60%), whereas the state-4 oxidation rate and $\Delta\psi$ were not affected by addition of malonate (less than 5% for the oxidation rate and 2% for $\Delta\psi$). Furthermore, the ADP/O ratios remained at their maximal values (data not shown).

The precedent results (Fig. 5) have shown that $\Delta\psi$ could not be directly related to the phosphorylation rates in all conditions, but an important parameter could be investigated: the changes in membrane potential between state 4 and state 3 (depolarization) (cf. Fig. 1). The meaning of this depolarization has been discussed by Zoratti et al. [20], but it is not established whether this depolarization is due only to the ADP/ATP exchange through the inner membrane or if it can be affected by changes in proton fluxes. Membrane depolarization has been analyzed by varying the different parameters of oxidative phosphorylation to determine the kinetic behavior of the proton fluxes during phosphorylation (Fig. 6).

In potato mitochondria the extent of depolari-

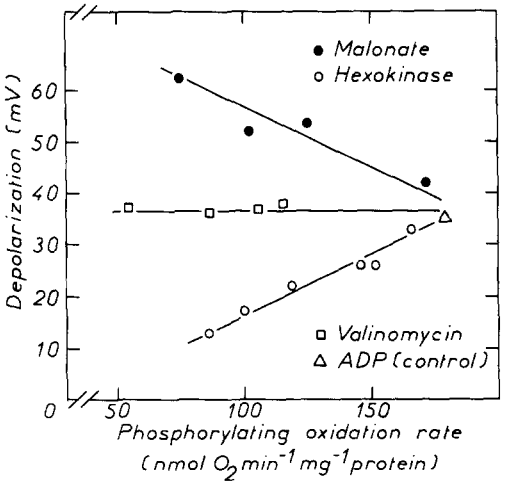

Fig. 6. Depolarization in state 3 obtained by varying the different parameters of oxidative phosphorylation (oxidation rate, phosphorylation rate and membrane potential). (●), effect of malonate; data from Fig. 5B. (○), effect of the hexokinaseglucose system; data from Fig. 5A. (□), effect of valinomycin, the phosphorylating oxidation rate was taken as the difference between the state-3 rate and the state-4 rate in the presence of valinomycin; data are taken from Fig. 4A. (△), effect of ADP, value of depolarization when ADP is added to purified mitochondria oxidizing succinate in the presence of ATP (control).

zation produced by phosphorylation was 35 mV for a state-3 oxidation rate of 180 nmol O_2/min per mg protein. When the oxidation rate was inhibited (malonate, cf. Fig. 5B) the extent of the depolarization was enhanced from 35 to 65 mV. In the presence of valinomycin, while the membrane potential and the phosphorylation efficiency were decreased, the depolarization between state 4 and state 3 remained unchanged. Finally, with the hexokinase-glucose system, the depolarization was decreased from 35 to 10 mV as the rate of phosphorylation was progressively diminished. The depolarization appeared to be very sensitive to the inhibition of the different parameters of oxidative phosphorylation and the representation of these results as $\Delta\psi$ vs. the oxidation rate shows that the relationship between $\Delta\psi$ and the electron flow was affected by the changes in proton fluxes.

Discussion

Using a TPP^+-sensitive electrode, the membrane potential in potato mitochondria oxidizing succinate was found to be about 220 mV. Between state 4 and state 3, the oxidation rate increases 3- to 4-fold (50–180 nmol O_2/min per mg protein), while $\Delta\psi$ changes are only 15–20% (230–190 mV) (see also Refs. 3 and 7). The results presented in this paper show that in this range of values a linear relationship exists between $\Delta\psi$ and the electron flow. The same relationship is obtained when mitochondria are uncoupled by CCCP, valinomycin or valinomycin + nigericin. While CCCP permeabilizes the inner mitochondrial membrane to protons and thus increases proton reentry, a direct effect on membrane permeability to protons could not be attributed to valinomycin. Hensley and Hanson [11] have shown the presence of an electroneutral H^+/K^+ antiporter in plant mitochondria which could explain the uncoupling effect of valinomycin. This antiporter is active in potato mitochondria, as a shrinking can be observed in the presence of limiting amounts of valinomycin in mitochondria-oxidizing succinate. This shrinking in a KCl medium, corresponding to a K^+ efflux from mitochondria, is sensitive to protonophores (CCCP). The influx of K^+ in energized mitochondria is mediated by the ionophore (valinomycin) and the efflux takes place via the H^+/K^+ antiporter which is sensitive to $\Delta\psi$ [17]. A K^+ cycling is created in the presence of valinomycin, associated with a proton reentry. This antiporter could explain the similar effects of CCCP and valinomycin, this effect can be attributed to an H^+ influx. Whatever the method used to collapse $\Delta\psi$ (protonophore or ionophore), the increase in oxidation rate is due to a modification of the membrane permeability to protons. It is suggested that the presence of the H^+/K^+ antiporter sensitive to $\Delta\psi$ [17] could account for the low effect of nigericin on potato mitochondria, as shown in Fig. 2A and by Ducet [7] and for the presence of low ΔpH [3]. Thus, in plant mitochondria, $\Delta\psi$ approaches the total protonmotive force ($\Delta\tilde{\mu}H^+$). Even though little variations of $\Delta\psi$ are concerned in the release of the oxidation rate, the above-mentioned results clearly show that a close relationship exists between $\Delta\psi$ and the oxidation rate in uncoupling conditions.

The constancy of the 'corrected ADP/O' when adding increasing concentrations of valinomycin show that the H^+ influx linked to the K^+ cycling induced by the conjugated effects of the ionophore and the antiporter is effectively nonphosphorylating and suggests that it remains at the same rate in the state-4 as in the state-3 respiration. Thus, the phosphorylation in the presence of valinomycin appears only driven by the additional H^+ ejection occurring in the phosphorylating state.

When the phosphorylation rate is inhibited (with carboxyatractyloside or with the hexokinase-glucose system), the relationship between $\Delta\psi$ and the oxidation rate is the same as in uncoupling conditions (Fig. 5A). These results differ from those previously reported for animal mitochondria (Ref. 6, see also Refs. 21 and 22). In plant mitochondria the availability of protons for the ATP synthase appears the same as for protonophores or the mitochondrial H^+/K^+ antiporter.

The study of the depolarization in different phosphorylating conditions gives additional information. The extent of the depolarization is very different depending on whether the proton efflux (oxidation) or the proton influx (phosphorylation) is limited. Depolarization in the phosphorylating state in the presence of valinomycin, even though membrane potential and phosphorylation efficiency are decreased, remains constant. When the

proton efflux (oxidation) is decreased (with malonate), depolarization increases. The limitation of the proton influx (with the hexokinaseglucose system) causes an opposite behavior of depolarization. While valinomycin has no effect on depolarization, the factors affecting the proton fluxes through the respiratory chain or through the ATP synthase modify this depolarization inversely. These results show that depolarization is not related to the rate of phosphorylation, and is not only the consequence of the ADP/ATP exchange during phosphorylation but also the result of a disequilibrium between the two opposite proton fluxes. The changes in the extent of depolarization fit well with the kinetic modifications of the proton fluxes, suggesting that no direct interaction exists between the respiratory chain and the ATP synthase in plant mitochondria.

The results presented in this paper show that the depolarization behaves as if the proton gradient is an intermediate between oxidation and phosphorylation. While low variations of $\Delta\psi$ are observed (Fig. 6) those which are seen are qualitatively significant and quantitatively sufficient to give the maximal rate of oxidation (Fig. 2) [9,23]. Furthermore, in contrast to recent results on Jerusalem artichoke mitochondria [3], these variations of membrane potential appear well correlated with the kinetics of proton fluxes as predicted by the chemiosmotic model [1]. The modifications of $\Delta\psi$ and oxidation rate induced by the operation of the ATP synthase are strictly equivalent to those produced by a protonophore. It is suggested that no preferential proton circuit exists between the ATP synthase and the respiratory chain and that a competition for the proton pool occurs between the ATP synthase and nonphosphorylating pathways. As a consequence, the oxidation rate appears to be dependent on the membrane potential, which in turn is modulated by changes in membrane permeability to protons, irrespective of whether these changes are caused by nonphosphorylating or phosphorylating proton pathways, as proposed by Nicholls [9] from hamster brown adipose tissue.

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