Control Over the Contribution of the Mitochondrial Membrane Potential ($\Delta \Psi$) and Proton Gradient ($\Delta p$) to the Protonmotive Force ($\Delta p$)

IN SILICO STUDIES

Revised for publication, March 27, 2008, and in revised form, July 8, 2008. Published, JBC Papers in Press, August 11, 2008, DOI 10.1074/jbc.M802404200

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The protonmotive force across the inner mitochondrial membrane ($\Delta p$) has two components: membrane potential ($\Delta \Psi$) and the gradient of proton concentration ($\Delta p$). The computer model of oxidative phosphorylation developed previously by Korzeniewski et al. (Korzeniewski, B., Noma, A., and Matsuoka, S. (2005) Biophys. Chem. 116, 145–157) was modified by including the K$^+$ uniport, K$^+$/H$^+$ exchange across the inner mitochondrial membrane, and membrane capacitance to replace the fixed $\Delta \Psi/\Delta p$ ratio used previously with a variable one determined mechanistically. The extended model gave good agreement with experimental results. Computer simulations showed that the contribution of $\Delta \Psi$ and $\Delta p$ to $\Delta p$ is determined by the ratio of the rate constants of the K$^+$ uniport and K$^+$/H$^+$ exchange and not by the absolute values of these constants. The value of $\Delta p$ is mostly controlled by ATP usage. The metabolic control over the $\Delta \Psi/\Delta p$ ratio is exerted mostly by K$^+$ uniport and K$^+$/H$^+$ exchange in the presence of these processes, and by the ATP usage, ATP/ADP carrier, and phosphate carrier in the absence of them. The K$^+$ circulation across the inner mitochondrial membrane is controlled mainly by K$^+$ uniport and K$^+$/H$^+$ exchange, whereas H$^+$ circulation by ATP usage. It is demonstrated that the secondary K$^+$ ion transport is not necessary for maintaining the physiological $\Delta \Psi/\Delta p$ ratio.

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According to the Mitchell chemiosmotic theory (1), the intermediate that couples the electron flow through the respiratory chain with ATP synthesis by ATP synthase in the mitochondrial matrix is the protonmotive force ($\Delta p$) across the inner mitochondrial membrane. This thermodynamic potential is composed of two components: electrical membrane potential ($\Delta \Psi$) and the difference between the cytosolic and matrix pH ($\Delta p$).

The usual measured value of $\Delta p$ is around 170–200 mV (2–9), although in older studies slightly higher values were encountered (10). This value may change depending on conditions, for instance with the intensity of ATP production by mitochondria. In the great majority of reports, the contribution of $\Delta \Psi$ to $\Delta p$ ($u = \Delta \Psi/\Delta p$) of 80–85% (0.8–0.85) was reported (2–10). This means that the value of $\Delta p$ is around 30 mV (0.5 pH units) (2–10) (again, in older reports, values around 50 mV are sometimes reported (10)). On the other hand, Bose et al. (11) reported a very low $\Delta p$ value, lower than 3 mV (0.05 pH units).

The contribution of $\Delta \Psi$ and $\Delta p$ to $\Delta p$ is very important from the kinetic point of view, because $\Delta \Psi$ and $\Delta p$ exert a different influence on such elements of the system and processes as: complex III (C3), complex IV (C4), ATP/ADP carrier, phosphate carrier (12), proton leak (13), and reactive oxygen species (ROS) production (8). The ATP/ADP carrier is driven by $\Delta \Psi$, whereas the phosphate carrier is driven by $\Delta p$. Complex III transfers four protons but only two positive charges, whereas complex IV transfers two protons and four positive charges (12); therefore complex III is relatively more sensitive to $\Delta p$, whereas complex IV is to $\Delta \Psi$. The proton leak seems to be more sensitive to $\Delta \Psi$ (13), while ROS production is to $\Delta p$ (8). Therefore, the total value of $\Delta p$ alone cannot define satisfactorily the kinetic properties of the oxidative phosphorylation system.

It is commonly accepted that the contribution of $\Delta \Psi$ and $\Delta p$ to $\Delta p$ is determined by secondary transport of ions, especially potassium ions (K$^+$), driven by building up the proton gradient across the inner mitochondrial membrane (12). It is also accepted that without this secondary transport, $\Delta p$ would be almost exclusively (about 99%) in the form of $\Delta \Psi$, because, at the real electrical capacity of the inner mitochondrial membrane and the pH buffering capacity of the matrix, the transport of a very small amount of protons would build up the physiological value of $\Delta p$ (12). It has been shown that $\Delta p$ and its components do not depend very significantly on the extramitochondrial [K$^+$] (5, 10), especially at higher physiological potassium ion concentrations. It has been observed in some studies (3, 10) that an increase in [P$i$] from 0 to physiological values (10 mm) causes a significant

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1 The abbreviations used are: C3, complex III; ROS, reactive oxygen species; i, intramitochondrial; e, extramitochondrial; DH, substrate dehydrogenation; C1, complex I; C4, complex IV; SN, ATP synthase; ANT, ATP/ADP carrier; PiT, phosphate carrier; cons, ATP consumption; LK, proton leak; Kuni, K$^+$ uniport; KHex, K$^+$/H$^+$ exchange; MCA, Metabolic Control Analysis.
decrease in Δp; at the same time ΔΨ slightly increases or remains constant after an initial decrease and ΔpH significantly decreases. On the other hand, Bose et al. encountered a significant increase in Δp and ΔΨ and a decrease in the very small ΔpH they measured.

Several trials of a quantitative description of the contribution of ΔΨ and ΔpH to Δp have been undertaken (14–16). These descriptions are based on different assumptions, give different predictions, and their verification by comparison with experimental data is limited. Generally, they are relatively simple and phenomenological. Recently Beard (17) developed a computer model of oxidative phosphorylation, based on the general structure of the model built by Korzeniewski et al. (18–22) that involves explicitly the K⁺ uniport, K⁺/H⁺ exchange across the inner mitochondrial membrane, and membrane capacitance. This model predicts a very low value of ΔpH (below 3 mV, 0.05 pH units), similar to that obtained in an experimental manner by Bose et al. (11).

The original model developed by Korzeniewski et al. (18–22) is able to reproduce, at least semi-quantitatively, a broad range of different kinetic properties of the oxidative phosphorylation system in isolated mitochondria and intact tissues. However, this model uses a simplified, phenomenological description of the relationship between Δp, ΔΨ, and ΔpH: it assumes a fixed, constant contribution of ΔΨ to Δp, u = 0.861. This assumption was based on experimental data concerning state 4, state 3, and intermediate states in isolated mitochondria (2), but is certainly oversimplified and unsatisfactory under many conditions.

Metabolic Control Analysis (MCA) (23–25) has appeared to be an immensely useful quantitative tool for analyzing the dynamic behavior of biochemical systems. It was used in a great number of experimental and theoretical studies concerning the control of metabolic pathways (see Refs. 19, 26–30 for a few examples).

THEORETICAL PROCEDURES

Computer Model—The computer dynamic model of oxidative phosphorylation in intact heart developed previously by Korzeniewski et al. (22) was extended to replace the fixed constant ΔΨ/Δp ratio (u = 0.861) with a more mechanistic description of ion transport across the inner mitochondrial membrane, similarly as it was done by Beard (17). The K⁺ uniport, K⁺/H⁺ exchange, and membrane capacitance were involved explicitly. The general scheme of the modeled system is presented in Fig. 1. The rates of K⁺ uniport (v_{Kuni}) and K⁺/H⁺ exchange (v_{KHex}) are described by the following kinetic Equations 1 and 2,

\[
v_{Kuni} = k_{Kuni} \cdot \left[ \frac{[K^+]_{e} \cdot e^{-\frac{\Delta \Psi}{R} \cdot T}}{[K^+]_{e} \cdot e^{-\frac{\Delta \Psi}{R} \cdot T}} \right] \quad (Eq. 1)
\]

\[
v_{KHex} = k_{KHex} \cdot \left[ \frac{[K^+]_{e} \cdot [H^+]_{e} - [K^+]_{e} \cdot [H^+]_{e}}{[K^+]_{e} \cdot [H^+]_{e}} \right] \quad (Eq. 2)
\]

where k_{Kuni} = 1.0 \times 10^{-5} \text{ min}^{-1} and k_{KHex} = 3.485 \times 10^{-3} \text{ min}^{-1} \mu\text{M}^{-1} are rate constants in the “reference point” (see below). R, T; and F have the typical meanings, subscript e means extramitochondrial and subscript i means intramitochondrial.

The changes over time in intramitochondrial and extramitochondrial [H⁺] are described by the following differential Equations 3 and 4,

\[
\frac{d[H^+]_e}{dt} = \frac{-4 \cdot v_{C1} - 2 \cdot v_{C3} - 4 \cdot v_{C4} + n_A \cdot v_{SM} + v_{Pi} + v_{LK} + v_{KHex}}{v_{Pi} + v_{LK} + v_{KHex}} \cdot \frac{R_{cm}}{\text{buff}_{Hi}} \quad (Eq. 3)
\]

\[
\frac{d[H^+]_i}{dt} = \frac{-4 \cdot v_{C1} + 4 \cdot v_{C3} + 2 \cdot v_{C4} - n_A \cdot v_{SM} - v_{Pi} - v_{LK} - v_{KHex}}{v_{Pi} - v_{LK} - v_{KHex}} \cdot \frac{1}{\text{buff}_{He}} \quad (Eq. 4)
\]

where R_{cm} = 3.35 is the ratio of the cell volume to mitochondria volume in heart cells, buff_{Hi} is the matrix proton-buffering coefficient, and buff_{He} is the cytosol proton-buffering coefficient. Some fraction ([P_{Pi}^0 - [P_{Pi}^-])/(P_{Pi}^0 + [P_{Pi}^-])] of P_{Pi}^- transported by phosphate carrier (PiT) to the mitochondrial matrix dissociates to P_{Pi}^- and H⁺, but they are quickly used by ATP synthase, and therefore no net protons are produced/consumed in the matrix. The opposite situation prevails in the cytosol. An analogous situation is the production/consumption of H⁺ (together with NADH) by the substrate dehydrogenation/complex I. All these processes are not related to proton and/or charge transfer across the inner mitochondrial membrane and therefore are not taken into account explicitly within the model. Complex I (C1) transports (for 2 electrons) 4 H⁺ from matrix to cytosol; complex III (C3) takes up (for 2 electrons) 2 H⁺ from the matrix, and releases 4 H⁺ to the cytosol; complex IV takes up (for 2 electrons or 1 oxygen atom) 4 H⁺ from the matrix (including 2 H⁺ for water molecule formation) and releases 2 H⁺ to the cytosol; ATP synthase (SN) transports n_A = 2.5 protons from cytosol to matrix for one ATP molecule synthesized; phosphate carrier (PiT), proton leak (LK), and K⁺/H⁺ exchange (KHex) transport 1 H⁺ from cytosol to matrix. All these processes are taken into account explicitly in the model.

The changes over time in intramitochondrial [K⁺] are described by the following differential Equation 5.

\[
\frac{d[K^+]_i}{dt} = (v_{Kuni} - v_{KHex}) \cdot R_{cm} \quad (Eq. 5)
\]

The potassium ion concentration in heart is very well regulated. (There are huge potassium stores in other tissues, especially in skeletal muscle, which can buffer the potassium concentration in heart (31).) Therefore, we assumed that the
extramitochondrial (cytosolic) K\(^+\) is constant. (This assumption also corresponds well to the isolated mitochondria system.) All the simulations presented below were performed under this assumption. However, we also tested the possibility that there is a constant cellular (cytosolic plus mitochondrial) pool of potassium ions and therefore Equation 6 applies.

\[ [K^+]_e = (v_{K_{\text{Hex}}} - v_{K_{\text{uni}}}) \]  

(Eq. 6)

In both cases the theoretical results were similar (not shown), and therefore the above assumption is not particularly important for the properties of the system.

The changes over time in \(\Delta \Psi\) are described by Equation 7,

\[ \Delta \Psi = \left( -4 \cdot v_{C_1} - 2 \cdot v_{C_3} - 4 \cdot v_{C_4} + \frac{R_m}{T_m} \right) \]  

(Eq. 7)

where the inner mitochondrial membrane capacitance, \(C_m\), is 1 \(\mu\text{M} \cdot \text{m}^{-2}\), as in Refs. 17 and 32. C1 transfers (for 2 electrons) 4 positive charges from matrix to cytosol; C3 transfers (per 2 electrons) 2 positive charges from matrix to cytosol (4 protons and 2 electrons); C4 transfers (for 2 electrons or 1 oxygen atom) 4 positive charges from matrix to cytosol (2 protons plus 2 electrons from cytosol to matrix); ATP synthase transfers \(n_A = 2.5\) positive charges from cytosol to matrix; ATP/ADP carrier, proton leak, and K\(^+\) uniport transport 1 positive charge from cytosol to matrix.

The reference point for the computer simulation carried out in the present study corresponded to a slowly beating intact heart (22). In this point, the following variable values were recorded: \(V_{O_2} = 2.633 \, \text{mmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}\), [ATP]\(_e\) = 6.663 mmol, [ADP]\(_e\) = 36.37 \(\mu\text{M}\), \([P_i]_e = 2.351 \, \text{mm}\), \(\Delta p = 181.9 \, \text{mV}\), \(\Delta \Psi = 156.6 \, \text{mV}\), \(\Delta pH = 25.30 \, \text{mV}\), \(u = \Delta \Psi/\Delta p = 0.861\), \([H^+]_e = 0.1 \, \text{mM}\), \([K^+]_e = 120 \, \text{mM}\), \([K^+]_i = 118.0 \, \text{mM}\). The rate constants of K\(^+\) uniport and K\(^+\)/H\(^+\) antiport were adjusted to be relatively quick and to give the \(\Delta \Psi/\Delta p\) value of 0.861 used in the original model (22): \(k_{K_{\text{uni}}} = 1.0 \times 10^{-5} \, \text{min}^{-1}\) and \(k_{K_{\text{Hex}}} = 3.485 \times 10^{-3} \, \text{min}^{-1} \cdot \mu\text{M}^{-1}\).

The complete description of the model of oxidative phosphorylation with a mechanistic description of the relationship between \(\Delta \Psi\) and \(\Delta pH\) in intact heart is located on the web site as supplemental material.

Metabolic Control Analysis—The most fundamental idea of MCA is based on the ratio of the relative change (d\(Y\)/\(Y\)) in some variable \(Y\) caused by a small relative change (d\(X\)/\(X\)) in some parameter/variable \(X\), to the latter change in Equation 8.

\[ \frac{\Delta Y/Y}{\Delta X/X} \]  

(Eq. 8)

In principle, these changes are infinitesimal, but in practice sufficiently small finite changes can be considered. In particular, control coefficients defined within MCA characterize the control exerted by particular components of a given metabolic system (enzymes, carriers, processes, and metabolic blocks) over different macroscopic properties (variables) of the system. Control coefficients are defined in Equation 9,

\[ C_U = \frac{\partial U/U}{\partial E/E} \]  

(Eq. 9)

where \(U\) is some property (variable) of the system and \(E_i\) is the activity/concentration of the \(i\)-th component of the system. Several control coefficients have been defined within MCA, concerning the control over the flux, metabolite concentrations, \(\Delta p\) etc. (see e.g. Refs. 7, 25, 28).

The metabolic control of particular elements of the oxidative phosphorylation system over \(\Delta p\), \(\Delta \Psi\), and \(\Delta pH\) was calculated according to Equation 9 (\(U\) represented \(\Delta p\), K\(^+\) cycling rate or \(H^+\) cycling rate). In subsequent computer simulations, the original rate constants of particular steps were increased by a relative factor of 0.01 (by 1%) and the relative changes in particular U-s between the original and the new steady state were recorded.

THEORETICAL RESULTS

First of all, our model predicts that in the reference point (that reflects the physiological conditions) there is a very small difference between the cytosolic and matrix potassium ion concentration (120.0 mm versus 118.0 mm, respectively) and \(\Delta \Psi\) is the dominant component of \(\Delta p\). These properties of the model agree excellently with recent conclusions drawn by Nicholls (33): “In an intact cell, or with isolated mitochondria in a physiologically relevant high K\(^+\) medium, there is essentially no gradient of K\(^+\) across the inner mitochondrial membrane, the concentration being close to 120 mm in each compartment. Under these conditions, \(\Delta pH\) is usually small . . .”.

To simulate the dependence of \(\Delta p\), \(\Delta \Psi\), and \(\Delta pH\) on \(k_{K_{\text{uni}}}\), the value of this constant was changed by six orders of magnitude, while \(k_{K_{\text{Hex}}}\) was kept constant. An increase in \(k_{K_{\text{uni}}}\) slightly decreases \(\Delta p\), as it can be seen in Fig. 2A. However, \(\Delta \Psi\) and \(\Delta pH\) change significantly with the increase in \(k_{K_{\text{uni}}}\): \(\Delta \Psi\) strongly decreases and \(\Delta pH\) strongly increases. At very low \(k_{K_{\text{uni}}}\) values, almost the entire \(\Delta p\) is in the form of \(\Delta \Psi\). An increase in \(k_{K_{\text{uni}}}\) elevates the mitochondrial \([K^+]\), but these changes are rather moderate: in the whole range of \(k_{K_{\text{uni}}}\) an about 2-fold increase in \([K^+]\) is observed.

The dependence of \(\Delta p\), \(\Delta \Psi\), and \(\Delta pH\) on \(k_{K_{\text{Hex}}}\) is opposite to the dependence on \(k_{K_{\text{uni}}}\) as it can be seen in the simulations shown in Fig. 2B. In these simulations, \(k_{K_{\text{Hex}}}\) was changed by six orders of magnitude, while \(k_{K_{\text{uni}}}\) was kept constant. An increase in \(k_{K_{\text{Hex}}}\) slightly increases \(\Delta p\), strongly increases \(\Delta pH\), and strongly decreases \(\Delta \Psi\). At very high \(k_{K_{\text{Hex}}}\) values, almost the entire \(\Delta p\) is in the form of \(\Delta \Psi\). \([K^+]\) drops about twice with the increase in \(k_{K_{\text{Hex}}}\).

The above simulations clearly show that the contribution of \(\Delta \Psi\) and \(\Delta pH\) to \(\Delta p\) strongly depends on the absolute values of the rate constants of the K\(^+\) uniport and K\(^+\)/H\(^+\) antiport: \(k_{K_{\text{uni}}}\) and \(k_{K_{\text{Hex}}}\). However, when the values of these constants are changed in parallel in relation to the reference point, by the same factor, and thus the ratio of the rate constants is kept constant \(k_{K_{\text{uni}}}/k_{K_{\text{Hex}}} = 2.896 \times 10^{-3}\) in the reference point), the value of \(\Delta p\) and the \(\Delta \Psi/\Delta p\) ratio are essentially unaffected. This is shown in Fig. 2C. Therefore, the contribution of \(\Delta \Psi\) and \(\Delta pH\) to \(\Delta p\) is determined by the relative values (ratio) of \(k_{K_{\text{uni}}}\) and \(k_{K_{\text{Hex}}}\). It is also worth noticing that \([K^+]\), remains constant.
Control over $\Delta \Psi / \Delta p$

The increase in [P$_i$] causes an increase in the rate of oxygen consumption and ATP synthesis (not shown) and an increase in the reduction level of cytochrome $c$ (see Fig. 4). This effect is not related to the activation of complex III by $p$, because such activation is not included in our model.

The absolute value of $\Delta p$ is controlled to the greatest extent by the ATP demand, although the control is distributed among essentially all components of the system. This is shown in Fig. 5. Generally, the processes that participate (directly or indirectly) in the production of $\Delta p$ (substrate dehydrogenation, complex I, complex III, and complex IV) have a positive control, while the processes dissipating $\Delta p$ (ATP usage, proton leak) have a negative control over $\Delta p$. The sum of the concentration control coefficients over $\Delta p$ equals zero (negative controls counter-balance positive controls), in agreement with the so-called connectivity property (25).

In the presence of K$^+$ uniport and K$^+/H^+$ antiport, just these two processes control to the greatest extent the contribution of $\Delta \Psi$ and $\Delta p$ to $\Delta p$. As can be seen in Fig. 6A, K$^+$ uniport has a great negative control over $u = \Delta \Psi / \Delta p$, while K$^+/H^+$ antiport has a great positive control over this variable. ATP usage has a moderate positive control over $u$, and the contribution of the remaining steps to the control is minor.

In the absence of K$^+$ uniport and K$^+/H^+$ antiport ($k_{\text{uni}}$ and $k_{\text{ex}}$ set to 0) the metabolic control over $u$ is exerted mainly by ATP usage and phosphate carrier (positive control) and ATP/ADP carrier (negative control) (Fig. 6B). Other components control this variable to a smaller extent. Under these conditions $u = 0.847$, which is similar to $u = 0.861$ in the presence of K$^+$ uniport and K$^+/H^+$ antiport. Therefore, the system is able to regulate effectively the contribution of $\Delta \Psi$ and $\Delta p$ to $\Delta \Psi$ even without the secondary K$^+$ transport.

The rate of K$^+$ cycling across the inner mitochondrial membrane is controlled mostly by the processes directly participating in this cycling: K$^+$ uniport and K$^+/H^+$ exchange. This is shown in Fig. 7. Both processes exert positive control of comparable size (the control by the $H^+/K^+$ exchange is somewhat higher). The $H^+$ cycling across the inner mitochondrial membrane is controlled to the greatest extent by ATP usage (not shown).

A decrease in the extramitochondrial phosphorylation potential brought about by an increase in ATP or [P] (with other external metabolite concentrations, i.e. [ATP] and [P] or [ATP] and [P], kept constant) leads to a decrease in $\Delta p$, while the increase in the extramitochondrial phosphorylation potential related to an increase in ATP (both ADP and P kept constant) is accompanied by an increase in $\Delta p$ (see Fig. 3, A–C). However, the relative contribution of $\Delta \Psi$ and $\Delta p$ to $\Delta p$ changes with changes in [ADP], [ATP], and [P]. While $\Delta \Psi$ follows the decrease in $\Delta p$ with an increase in [ADP] and the increase in $\Delta p$ with an increase in [ATP], $\Delta p$ slightly increases in the first case and slightly decreases in the second. An even more interesting situation takes place in the case of an increase in [P$_i$], here at higher P$_i$ concentrations $\Delta \Psi$ slightly increases (after an initial decrease), at the cost of a significant decrease in $\Delta p$. Therefore, the contribution of $\Delta \Psi$ to $\Delta p$ increases of course at higher [P$_i$]. The simulated dependence of $\Delta p$, $\Delta \Psi$, and $\Delta p$ on [P] is compared in Fig. 3C with experimental data by Kunz et al. (3) and Nicholls (10). Generally, a good agreement can be observed (see “Discussion” for details). In our simulations, $\Delta p$, $\Delta \Psi$, and $\Delta p$ are essentially independent of the extramitochondrial K$^+$ concentration (Fig. 3D) (in this theoretical analysis, different constant [K$_i$], in the range between 0 and 120 mM were fixed in subsequent simulations) although at low [K$_i$] the system approaches the steady state very slowly (starting from the reference point) because the K$^+$ uniport and K$^+/H^+$ exchange are very slow (not shown). Our simulations are compared in Fig. 3D with experimental data by Czyz et al. (5). Again, a good agreement can be observed (see also “Discussion”).

The absolute value of $\Delta p$ is controlled to the greatest extent by the ATP demand, although the control is distributed among essentially all components of the system. This is shown in Fig. 5. Generally, the processes that participate (directly or indirectly) in the production of $\Delta p$ (substrate dehydrogenation, complex I, complex III, and complex IV) have a positive control, while the processes dissipating $\Delta p$ (ATP usage, proton leak) have a negative control over $\Delta p$. The sum of the concentration control coefficients over $\Delta p$ equals zero (negative controls counter-balance positive controls), in agreement with the so-called connectivity property (25).
Control over ΔΨ/ΔpH

simulations presented in Fig. 2 clearly demonstrate that it is not the absolute values of the rate constants of K⁺ uniport and K⁺/H⁺ antiport (k_{Kuni} and k_{KHex}, respectively), but the ratio of these rate constants that determines the value of parameter u = ΔΨ/Δp. Significant variations in either k_{Kuni} or k_{KHex} when the other rate constant remains unchanged, significantly affect the contribution of ΔΨ and ΔpH to Δp (Fig. 2, A and B). On the other hand, an increase in both rate constants by six orders of magnitude has only an insignificant effect on Δp, ΔΨ, and ΔpH when the k_{Kuni}/k_{KHex} ratio is fixed at 2.896×10⁻³.

Extramitochondrial ADP decreases and extramitochondrial ATP increases Δp and ΔΨ, whereas a small opposite effect on ΔpH is observed (Fig. 3, A and B). Such a behavior of the system is not surprising, because a high external [ADP] activates the ATP/ADP carrier, accelerates the dissipation of ΔΨ and thus increases the contribution of ΔpH to Δp, while a high external [ATP] has the opposite effect. However, one must bear in mind that the potential effect of [ATP] on the mitoK_{ATP} channel is not included in our simple model. On the other hand the half-inhibiting [ATP] for this channel is very low, in the micromolar range (34).

An increase in the extramitochondrial [P_i] decreases Δp and ΔpH, but slightly increases (at higher concentrations) ΔΨ. This simulated behavior of the system is generally similar to the experimental results obtained by Nicholls (10) and Kunz et al. (3), as shown in Fig. 3C. The contribution of ΔpH to Δp was probably somewhat overestimated in Ref. 10, because small amounts of valinomycin were used in that study to allow Rb⁺ flow across the inner mitochondrial membrane; valinomycin dissipates ΔΨ and thus builds up ΔpH. In the more recent study (3), DDA⁺ that does not need valinomycin was used to determine ΔΨ.

On the other hand, Bose et al. (11) observed a significant (much higher than in our simulations, but the qualitative tendency is similar) P_i-induced increase in ΔΨ, but they measured a very low value of ΔpH, below 3 mV (0.05 pH units), which is not what was usually observed by other investigators (2–10). It is not clear if these differences are due to different experimental systems and conditions used (resulting in e.g. different values of k_{Kuni} and/or k_{KHex}) or different methods of ΔΨ and (especially) ΔpH determination.

Our simulations predict that Δp, ΔΨ, and ΔpH are essentially independent of extramitochondrial K⁺ concentration (Fig. 3D). These theoretical predictions are generally similar to the experimental data obtained by Czyż et al. (5), although the authors observed that ΔΨ slightly increases and ΔpH slightly increases at low, unphysiological [K⁺]_e. A similar behavior of the system was encountered by Nicholls (10). Of course this (rather insig-

**FIGURE 3.** Simulated dependence of Δp, ΔΨ, and ΔpH on extramitochondrial (ADP) (A), (ATP) (B), [P_i] (C), and [K⁺] (D). Thick lines without points, computer simulations; points connected with thin lines, experimental data; solid lines, Δp; dotted lines, ΔΨ; dashed lines, ΔpH; full symbols in C, data from Ref. 3; empty symbols in D, data from Ref. 5.

**FIGURE 4.** Simulated dependence of the reduction level of cytochrome c on extramitochondrial [P_i].

**FIGURE 5.** Simulated control coefficients of particular components of the system over Δp.

**DISCUSSION**

One of the most important findings of the present study is that the contribution of ΔΨ and ΔpH to Δp is determined by the relative activity of the K⁺ uniport and K⁺/H⁺ antiport. The
significant, considering low [K+] difference between model predictions and experimental data can be caused by the fact that within the model, the kinetic description of K⁺ uniport and K⁺/H⁺ antiport is oversimplified and does not work properly at very low [K⁺]. Another possibility is that, as discussed above, the system approaches very slowly a steady-state at low [K⁺], and therefore the steady-state had not been reached in the discussed experimental studies. Indeed, computer simulations demonstrated that a dependence of ΔΨ and ΔpH on [K⁺] similar to that seen in Refs. 5, 10 can be easily obtained if the values of ΔΨ and ΔpH from the transient state preceding the steady state are plotted in the diagram (not shown).

The decrease in the contribution of ΔpH to Δp with the increase in [P_i] from 0 to physiological values can be explained as follows. The phosphate carrier carries out the transport of P_i from cytosol to the mitochondrial matrix coupled with the transport of H⁺ to the matrix. Therefore, fast action of this carrier causes intensive ΔpH dissipation. At low P_i concentrations, this carrier is inhibited, and therefore the dissipation of ΔpH is restricted, which leads to a relatively higher contribution of ΔpH to Δp. An increase in [P_i] abolishes this effect and causes a decrease of the ΔpH contribution and thus an increase in the ΔΨ contribution to Δp.

Fig. 4 shows that an increase in [P_i] from 0 to physiological values results in an increase in the reduction level of cytochrome c. It has been postulated by Bose et al. (11) that the P_i-induced increase in ΔΨ and cytochrome c reduction level constitutes evidence for a direct activation of complex III of the respiratory chain by extramitochondrial inorganic phosphate. However, the present studies do not confirm this proposition, because both effects can be accomplished by a computer model of oxidative phosphorylation that does not involve the possible activation of complex III by P_i (although we have to admit that the simulated increase in ΔΨ is very small).

The model developed by Beard (17) predicts that almost the entire Δp is in the form of ΔΨ, and therefore ΔpH is very low, in accordance with the experimental results obtained in Ref. 11. However, this prediction is simply a consequence of the assumption that there is no passive potassium flux (k_{Kuni} = 0) and that the K⁺/H⁺ exchange is very active (17). If a lower rate constant of the K⁺/H⁺ exchange is assumed, along with a nonzero rate constant of K⁺ uniport, as is done in the present study, a much higher value of ΔpH of about 30 mV is obtained, in agreement with the great majority of experimental data coming from different laboratories (2-10).

It is not surprising that ATP usage has the greatest (negative) control over the absolute value of Δp (Fig. 5), because ATP usage is the main process determining ATP turnover. An increase in the intensity of this process leads to a decrease in the cytosolic phosphorylation potential. This decrease is transferred (through the ATP/ADP carrier and phosphate carrier) to the mitochondrial phosphorylation potential and then (through ATP synthase) to the proton motive force (Δp). The proton leak dissipates Δp, and therefore it also has a negative control. However, the fraction of protons returning to the matrix by this process is relatively small (17% in a slowly beating heart within the model), which results in a relatively small control coefficient.

The presented theoretical results agree very well with the experimental determination of the control of oxidative subsystem (substrate dehydrogenation, complex I, complex III, and complex IV), phosphorylation subsystem (ATP synthase, ATP/ADP carrier, phosphate carrier, ATP usage), and proton leak subsystem over Δp carried out within the frame of the ‘top-down approach’ to MCA (7, 35).

In the presence of K⁺ uniport and K⁺/H⁺ antiport, the contribution of ΔΨ to Δp (parameter u) is mainly controlled just by these processes (Fig. 6A). The magnitude of the control is identical for both processes, but has the opposite sign. The K⁺ uniport (leak) dissipates ΔΨ and therefore exerts a negative control over u = ΔΨ/Δp, whereas the K⁺/H⁺ uniport dissipates ΔpH and therefore its control over u is positive. The equal
Control over $\Delta \Psi / \Delta pH$

in size but oppositely-directed control exerted by the $K^+$ uniport and $K^+/H^+$ explains why the contribution of $\Delta \Psi$ and $\Delta pH$ to $\Delta p$ remains essentially unaffected when the activities (rate constants) of both processes are changed in parallel by the same relative factor (Fig. 2C).

Our model predicts that the system seems to be able to regulate sufficiently the contribution of $\Delta \Psi$ and $\Delta pH$ to $\Delta p$ even without the secondary $K^+$ transport. The simulations shown in Fig. 6B explain the basis of this surprising system property. Namely, in the absence of $K^+$ circulation, the control over $u$ is taken over by ATP usage, ATP/ADP carrier, and phosphate carrier. ATP usage had already a significant control in the presence of the $K^+$ uniport and $K^+/H^+$ antiport, because a decrease in $\Delta p$ causes an increase in $u$. On the other hand, in the absence of the secondary $K^+$ transport, the ATP/ADP carrier and phosphate carrier seem to take over the role of $K^+$ uniport and $K^+/H^+$ antiport in determining the $\Delta \Psi/\Delta pH$ ratio. This is logical, because the ATP/ADP carrier dissipates $\Delta \Psi$ (as the $K^+$ uniport) and the phosphate carrier dissipates $\Delta pH$ (as the $K^+/H^+$ antiport).

It is also noteworthy that the obtained simulation results do not depend qualitatively on the value of membrane capacitance $C_m$, because changing this parameter within several orders of magnitude has only a minor influence on the distribution of $\Delta \Psi$ between $\Delta \Psi$ and $\Delta pH$ (1% relative change in the value of parameter $u$) without a significant change in $\Delta p$ (results not shown).

The rate of $K^+$ circulation across the inner mitochondrial membrane is mostly controlled by $K^+$ uniport and $K^+/H^+$ antiport (Fig. 7). The control exerted by the latter is somewhat greater, because the former is very sensitive to the dominant component of $\Delta p$: $\Delta \Psi$, appearing in the power expression (Equation 1). According to the Metabolic Control Analysis paradigm, the greater the sensitivity to metabolite concentrations (or thermodynamic forces), the lower the control over the flux, and the inverse (25).

Of course, the model developed in the present study contains several simplifications. A simple kinetics (linear dependence of rates on ion concentrations) of the $K^+$ uniport and $K^+/H^+$ exchange was assumed. Constant cytosolic and mitochondrial free $[Mg^{2+}]$ was assumed; this assumption is justified by the presence of a significant magnesium binding pool in both compartments (36) and by the essentially constant $[ATP]$ (changes in $[ATP]$ caused by AMP deamination are potentially the greatest source of short-term variations in $[Mg^{2+}]$). It is also widely accepted, that the matrix $[Mg^{2+}]$ modulates $K^+/H^+$ antiport activity. Nevertheless, under physiological magnesium concentrations, exchanger inhibition is reported to be already saturated, and moderate $[Mg^{2+}]$ changes should not significantly affect exchanger turnover rate (37). It was assumed that the mitochondrial matrix volume is substantially constant in intact tissues. (This is certainly not the case in isolated mitochondria.) Nevertheless, it seems unlikely that the general theoretical results obtained in the present study depend significantly on these assumptions.

The present theoretical study is important for understanding the functioning of the system. As mentioned in the Introduction, various oxidative phosphorylation complexes, proton leak, and free radical production are differentially regulated by $\Delta \Psi$ and $\Delta pH$. The distribution of control over $u = \Delta \Psi/\Delta pH$ among particular oxidative phosphorylation complexes may be particularly important in pathological conditions, especially in the case of inborn enzyme deficiencies (causing mitochondrial diseases, Ref. 38), where an elevated free radical production is one of the most important pathogenic factors.

Summing up, in the present study the computer model of oxidative phosphorylation developed previously by Korzeniewski et al. was extended by including explicitly the $K^+$ uniport, $K^+/H^+$ exchange, and capacitance of the inner mitochondrial membrane. Computer simulations demonstrated that the $\Delta \Psi/\Delta pH$ ratio is mainly determined by the relative activities (rate constants) of the $K^+$ uniport and $K^+/H^+$ exchange rather than by the absolute values of these activities. It was shown that the absolute value of $\Delta p$ is mainly controlled by ATP usage, although the control is distributed among essentially all components of the system. The model predicted that in the presence of the $K^+$ uniport and $K^+/H^+$ antiport, the metabolic control (defined according to the Metabolic Control Analysis paradigm) over the contribution of $\Delta \Psi$ to $\Delta p$ ($u = \Delta \Psi/\Delta p$) is exerted mainly by these processes, while in the absence of them, by ATP usage, ATP/ADP carrier, and $P_i$ carrier. It is shown that $K^+$ circulation across the inner mitochondrial membrane is controlled by $K^+$ uniport and $K^+/H^+$ antiport. Finally, it was postulated that the system is able to control effectively the physiological $\Delta \Psi/\Delta pH$ ratio even without the secondary $K^+$ transport system.

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