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Quantification of the relative contribution of parallel pathways to signal transfer: application to cellular energy transduction

Bernard Korzeniewski[a,*], Guy C. Brown[b]

[a] *Institute of Molecular Biology, Jagiellonian University, al. Micjkiewicza 3, 31-120 Kraków, Poland*
[b] *Department of Biochemistry, University of Cambridge, Cambridge, UK*

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Abstract

A simple mathematical formalism designed to quantify the relative contribution of parallel pathways to signal transduction is presented and applied to the regulation of the respiration rate by ATP, ADP and P_i concentrations in response to an increase of energy demand in isolated mitochondria. Theoretical studies were performed by means of the computer model of oxidative phosphorylation developed previously. Many earlier experimental studies have shown that externally-manipulated concentrations of all three metabolites can influence the respiration rate significantly. However, the effect of changes in [ATP], [ADP] and [P_i] that actually take place during an increased energy demand have not been determined in a quantitative way. It was shown in the present paper that [ADP] is the main regulatory factor which stimulates respiration during transition from state 4 to state 3 imposed by an addition of increasing amounts of an artificial ADP-regenerating system. Changes in [ATP] and [P_i] contribute to the respiration rate increase very weakly, and only in the nearest neighbourhood of state 3. Generally, changes in [ADP] are responsible for approx. 90% of the respiration rate increase during the state 4 → state 3 transition, while the remaining approx. 10% is due to changes in [P_i] and [ATP].

Keywords: Signal transduction; Oxidative phosphorylation; Regulation; Computer model; Energy demand; Isolated mitochondria

1. Introduction

In the last 20 years, there has been an explosive growth in our knowledge of signal transduction pathways within cells. However, this knowledge is almost exclusively qualitative, rather than quantitative, and this leads to great difficulty in predicting or explaining cellular behaviour when more than one signal transduction pathway is involved. One common problem requiring quantitative treatment is to assess the relative contribution of different signal transduction pathways to a partic-

* Corresponding author. Tel.: +48 12 634 13 05 ext. 203; fax: +48 12 633 69 07; e-mail: benio@mol.uj.edu.pl

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ular cellular response to a hormone or other change. We develop here a simple quantitative approach to this question, and apply it to a long-standing problem in cellular signalling and energy transduction: the relative contributions of changes in ATP, ADP, and P_i to the stimulation of mitochondrial ATP production by ATP consumption.

The main process in the animal cell responsible for the production of energy in the form of ATP is oxidative phosphorylation in mitochondria. The rate of ATP utilisation can change in time, depending on conditions. Mitochondria can respond to an increase in energy demand, by increasing the rates of respiration and ATP synthesis. In suspension of isolated mitochondria, the increased energy demand can be imitated by an addition of increasing amounts of hexokinase in the presence of glucose (an artificial ADP-regeneration system) which leads to a subsequent increase in oxygen consumption and ATP synthesis, until mitochondria become saturated. This is what is called the transition from state 4 (no hexokinase added) to state 3 (saturating amount of hexokinase).

A question arises how mitochondria are 'informed' about an increased energy demand. Oxidative phosphorylation in mitochondria communicates kineticly with the ATP usage system via the concentration of ATP as well as via the concentrations of ATP hydrolysis products, namely: ADP and inorganic phosphate. Plenty of work was devoted to the problem of which of these metabolites regulate respiration and to what extent [1–16]. It has been originally proposed by Chance and Williams [1] that ADP concentration constitutes the relevant feedback signal. On the other hand, concentrations of all three metabolites change at an increased energy demand (ATP consumption) and therefore each of these metabolites could, at least potentially, regulate the respiration rate. It was indeed observed that different, externally imposed, combinations of ATP, ADP and P_i concentrations give different values of oxygen consumption by suspension of isolated mitochondria [2,3]. This prompted some authors to postulate that the ATP/ADP ratio [4,5], ATP/ADP* P_i ratio [6], phosphorylation potential [7,8] or Atkinson's energy charge [9] is the relevant regulatory parameter. However, the situation where the concentrations of ATP, ADP and P_i are arbitrarily fixed and/or changed by an experimentator does not fully reflect the physiological situation. In such experiments, it was only possible to state what is the sensitivity of mitochondria to particular metabolites, but it was not possible to determine how the metabolite concentrations change as energy demand changes. The concentrations of ATP, ADP and P_i are not related to each other randomly during an increased energy demand (for example imposed by an addition of increasing amounts of hexokinase); instead, a unique relationship exists between them. The influence of particular metabolite concentration changes on the respiration rate depends not only on the sensitivity of oxidative phosphorylation to a given metabolite concentration, but also on the concentration changes that actually take place during physiological regulation. The experimental studies focused mainly on the former factor, while the latter factor has not been analysed in a quantitative way to a satisfactory extent. Therefore a still current problem is which metabolites, and to what extent, regulate oxidative phosphorylation at the concentration changes taking place during the state 4 → state 3 transition.

In the present article the above-formulated problem is studied by means of the dynamic computer model of oxidative phosphorylation in muscle mitochondria developed previously and successfully tested for large-scale changes in fluxes and metabolite concentrations [17]. The problem of quantifying signal transfer via parallel pathways is analysed within the framework of Signal Transfer Analysis [18], but the theory is further developed below to address this type of problem.

2. Theory

A signal (S) may cause some cellular response in a target (T). The signal may be a molecule, such as a hormone, or a rate, with steady-state level or rate S, and a change in this signal (ΔS) may cause a change in the steady-state level or rate of some target molecule or rate (ΔT) within a cell or tissue. For example, a change in the

steady-state level of some hormone may act as a signal (S) to cause a change in the steady-state rate of transcription of some gene, which we are regarding as the target (T). Now we can quantify the steady-state response of the target to the signal as a response coefficient (R):

$$R(S,T) = \mathrm{d}T/\mathrm{d}S \tag{1}$$

where the change in S is extrapolated to infinitesimally small change, so the response coefficient is a measure of the sensitivity of T to changes in S only for one particular level of S and one set of conditions.

Alternatively the response coefficient can be defined in fractional form as in [18]:

$$R(S,T) = (\mathrm{d}T/T)/(\mathrm{d}S/S) \tag{2}$$

but we will be using the non-normalised form of the response coefficient Eq. (1) in the rest of this paper.

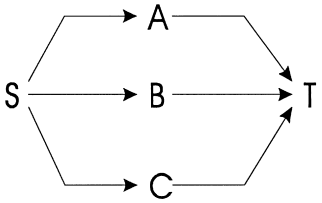
Now this target response to the signal may be mediated by more than one signal transduction pathway; for example a hormone may act at several different receptors to cause a change in gene transcription. Let each of these pathways pass through a different signalling intermediate M (e.g. A, B, and C in Scheme. 1).

Then the problem we wish to address is what fraction of the target response to the signal is mediated by each of these pathways. We may define this fraction [$F(M)$] as:

$$F(M) = (\mathrm{d}T/\mathrm{d}S)_M/(\mathrm{d}T/\mathrm{d}S) \tag{3}$$

where:

$$(\mathrm{d}T/\mathrm{d}S)_M = [\partial T/(\partial M/M)]\cdot[(\mathrm{d}M/M)/\mathrm{d}S] \tag{4}$$



Scheme 1.

and

$$\mathrm{d}T/\mathrm{d}S = \sum_M (\mathrm{d}T/\mathrm{d}S)_M \tag{5}$$

and $M = A$, B or C.

Eq. (4) quantifies the response of the target to the signal mediated through a particular intermediate (M), and shows that this response is made up of two components: the sensitivity of the intermediate (M) to the signal (i.e. how much M changes when S changes), and the sensitivity of the target to the intermediate (i.e. how much T changes when M alone changes). Eq. (5) asserts that the total response of T to S is the sum of the partial responses mediated by each of the parallel pathways. Eq. (3) quantifies the fraction of the total response mediated by a particular pathway.

The responses quantified above are differential and refer to very small or strictly infinitesimal signal change from the reference state. However, we may often be interested in the response to a finite change in signal, for example the response to changing the level of a hormone from zero to some particular level. When S changes from x to y we have:

$$\begin{aligned}\Delta T(S=x,y) &= \int_{S=x}^{y} R(S,T)\cdot \mathrm{d}S \\ &= \int_{S=x}^{y} (\mathrm{d}T/\mathrm{d}S)\cdot \mathrm{d}S\end{aligned} \tag{6}$$

This integral response of T to S can be decomposed and analysed in terms of the response of each of the intermediate pathways to S, and the response of T to these intermediates, by integrating Eqs. (4),(5):

$$\begin{aligned}\Delta T(S=x,y) &= \sum_M \int_{S=x}^{y} [\partial T/(\partial M/M)] \\ &\quad /[(\mathrm{d}M/M)/\mathrm{d}S]\cdot \mathrm{d}S \\ &= \sum_M \Delta T_M(S=x,y)\end{aligned} \tag{7}$$

It is important to note that the responses of T to the intermediates ($\partial T/(\partial M/M)$) are partial

differentials, i.e. they quantify the response of T to M when all intermediates of other pathways from S to T are held constant.

Eq. (7) encapsulates and expresses the integral response of T to S in terms of the various pathways involved, and enables us to quantify what fraction of the integral response of T to S is due to each of the intermediate pathways:

$$F(M) = \Delta T_M / \Delta T \tag{8}$$

In the following considerations, signal S is energy demand (concentration/rate constant of hexokinase), target T is the respiration rate, and different signal-transducing metabolites M (A, B or C) correspond to ATP, ADP and P_i.

3. Modelling procedures

In the model of oxidative phosphorylation in isolated skeletal muscle mitochondria respiring on pyruvate used for simulations, each reaction (group of reactions) and process, taken into account explicitly, was described by an appropriate kinetic equation, expressing the dependence of the reaction rate on different metabolite concentrations. The kinetic scheme of reactions of the oxidative phosphorylation system which is modelled is presented in Fig. 1. The model of this system takes into account explicitly the following steps of oxidative phosphorylation: substrate dehydrogenation (hydrogen supply to the respiratory chain), complex I, complex III, complex IV (cytochrome oxidase), ATP synthase, ATP/ADP carrier, artificial ATP usage system (hexokinase + glucose) and adenylate kinase. The rates of changes in time of the metabolite concentrations were expressed as a set of ordinary differential equations. This set was solved numerically, with an aid of a computer. The kinetic description of the model is given elsewhere [17].

In the simulations performed in the present work, an increase in energy demand was equivalent to an increase in the concentration (rate constant) of hexokinase. At each value of energy demand, the steady-state values of the oxygen consumption flux and metabolite (ATP, ADP and P_i) concentrations were recorded. The energy demand (hexokinase concentration) was equal to zero in state 4. When increasing concentrations (rate constants) of hexokinase were introduced, subsequent increases in the respiration rate took place. Mitochondria became saturated (no further significant increase in the respiration rate) at energy demand equal to approx. 100 (standarized value) in arbitrary units. However, some changes in metabolite concentrations could be still observed when energy demand achieved 150. The respiration rate was standarized to be equal to 100 (in arbitrary units) in state 3.

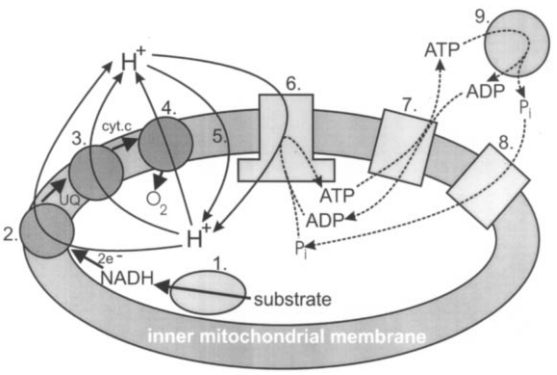


Fig. 1. Scheme of the modelled system —oxidative phosphorylation in isolated mitochondria. (1) substrate dehydrogenation; (2) complex I; (3) complex III; (4) complex IV (cytochrome oxidase); (5) proton leak; (6) ATP synthase; (7) ATP/ADP carrier; (8) phosphate carrier; and (9) artificial ATP usage sytem (hexokinase + glucose). It can be seen that ATP usage interacts with the ATP production system in mitochondria via concentrations of three metabolites: external ATP, ADP and P_i.

In order to compare the total effect of changes in [ATP], [ADP] and [P_i] taken together with the separate effects of particular metabolite concentrations on the respiration rate at different energy demands, a small increase in energy demand (by 0.1 arbitrary units) was performed at a given energy demand. This resulted in a small increase in the respiration caused by changes in [ATP], [ADP] and [P_i]. $R(S,T)$ was calculated according to Eq. (1). Next, the effect of only one metabolite concentration change (with the two remaining metabolite concentrations kept constant) on the respiration rate was simulated for all three metabolites. $[\partial T/(\partial M/M)]$ and $[(\mathrm{d}M/M)/\mathrm{d}S]$ were calculated on the basis of the recorded

changes in the respiration rate as well as in [ATP], [ADP] and [P_i]; $(dT/dS)_M$ was calculated according to Eq. (4). Of course, the separate effects of particular metabolite concentrations summed up to the total effect of all metabolite concentrations acting together Eq. (5). The described procedure was repeated several times for different values of energy demand.

4. Theoretical results and discussion

The simulated dependences of the respiration rate as well as of concentrations of ATP, ADP and inorganic phosphate on energy demand (hexokinase concentration) are presented in Fig. 2. The effect of energy demand on the oxygen consumption flux is very similar to the dependence obtained experimentally [19]. It can be seen that respiration increases near-linearly with hexokinase concentration until energy demand reaches a saturating value equal to 100 (in arbitrary units). A further increase in energy demand does not cause any significant stimulation of respiration. Therefore the range of energy demand between 0 (state 4) and 100 (state 3) can be regarded as a 'regulatory region', relevant to the physiological regulation of oxidative phosphorylation by [ATP], [ADP] and [P_i] in response to varying energy demand.

The potential efficiency of particular metabolites as regulatory factors depends on the relative changes in their concentrations during the transition from state 4 to state 3. These changes are also presented in Fig. 2. It can be easily seen that only ADP experiences significant relative changes in concentration in the whole range of the 'regulatory region'. [ATP] and [P_i] are essentially constant in most of this range, changing markedly only in the near vicinity of the onset of state 3 (energy demand equal to 100). At energy demands higher than necessary for reaching state 3, [ADP] begins to diminish because of the shift in adenylate kinase reaction equilibrium towards AMP production. This is related to a significant fall in the ATP concentration behind the 'saturation point'. Two different steady states with the same ADP concentration, but slightly different respiration rates, can exist since the concentrations of ATP and AMP (and P_i) are different in these steady states (the equilibrium equation for adenylate kinase, describing the interrelationship between [ATP], [ADP] and [AMP], has two different solutions for one ADP concentration).

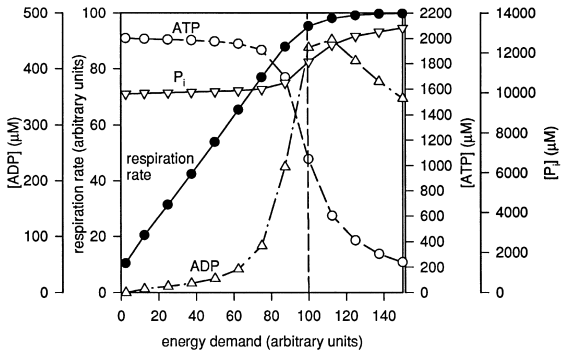


Fig. 2. Simulated dependences of the respiration rate as well as [ATP], [ADP] and [P_i] on energy demand (hexokinase concentration). Energy demand equal to 0 corresponds to state 4 and energy demand equal to 100 corresponds to the onset of state 3. The dashed line indicates the upper limit of the regulatory region.

High relative changes in the ADP concentration during state 4 → state 3 transition suggest that this metabolite is the main factor adjusting the rate of ATP synthesis to an increased energy demand in isolated mitochondria. However, the efficiency of a given metabolite as a regulator depends not only on relative changes in the metabolite concentration, but also on the sensitivity of mitochondria (as whole entities) to this concentration. The 'regulatory power' of a metabolite M $[(dT/dS)_M]$ in a given state (at a given energy demand) can be defined as a relative change in $[M]$ (during transition to an adjoining steady state, caused by a small, determined increase in energy demand) $((dM/M)/dS)$ multiplied by the sensitivity of mitochondria to $[M]$ in this state $[\partial T/(\partial M/M)]$ (compare Eq. (4)). The regulatory power of M is equivalent to the partial response of T to S transduced via M (Eq. (4)).

The increase in the respiration rate during state 4 → state 3 transition is the result of the effect of the increase in [ADP] and [P_i] as well as of the decrease in [ATP]. The regulatory powers of these metabolites (partial target responses to the signal mediated by these metabolites) at dif-

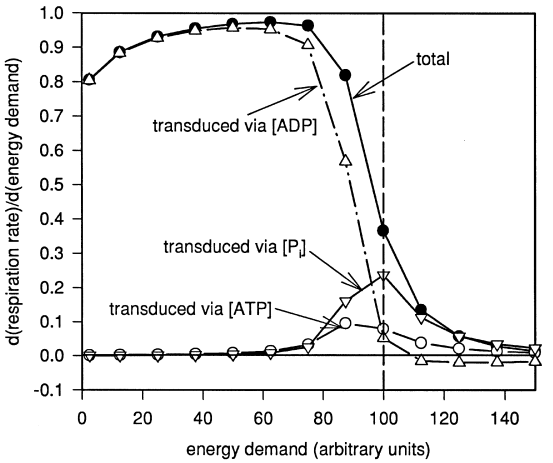


Fig. 3. Relative regulatory powers of ATP, ADP and P_i at different energy demands. Total response of the respiration rate to energy demand (dT/dS) is compared with the responses transduced via particular metabolites [$(dT/dS)_M$]. The dashed line indicates the upper limit of the regulatory region.

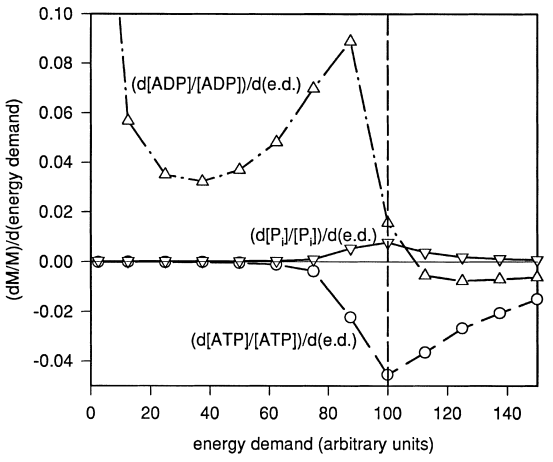


Fig. 4. Relative changes in the concentrations of ATP, ADP and P_i caused by a small increase in energy demand [$(dM/M)/dS$] at different energy demands. The dashed line indicates the upper limit of the regulatory region. E.d. = energy demand.

ferent energy demands are presented in Fig. 3 and compared with the total regulatory power of all three metabolites acting together (total target response to the signal), equal to the sum of the regulatory powers of particular metabolites. The y axis represents here the change in the respiration rate (in arbitrary units), caused by a change in a particular metabolite concentration, which in turn is caused by a small increase in energy demand by 0.1 arbitrary units in a given state, divided by this energy demand change [$(dT/dS)_M$]. Fig. 3 clearly shows that ADP is essentially the only regulator of oxidative phosphorylation in almost entire regulatory region. Additionally, the regulatory power of ADP is similar throughout most of this range. This is because, although mitochondria are less sensitive to ADP concentration at higher energy demands (saturation of oxidative phosphorylation with higher [ADP]), the changes in [ADP] are adequately larger at higher energy demands and the product of changes and sensitivities remains approximately constant.

Changes in ATP and inorganic phosphate concentrations exert a small effect on the respiration rate only in the near neighbourhood of the onset of state 3. After an integration of the areas under particular curves (Eqs. (6) and (7)) one obtains that the change in [ADP] is responsible for 88% of the respiration rate stimulation during state 4 → state 3 transition [$F(\mathrm{ADP}) = 0.88$], while changes in [ATP] and [P_i] account for only, 4 and 8% respectively, of this stimulation [$F(\mathrm{ATP}) = 0.04$, $F(P_i) = 0.08$] (Eq. (8)).

To study the origin of the high relative regulatory power of ADP, we simulated changes in metabolite concentrations as well as sensitivities of respiration flux to metabolite concentrations at different energy demands. The dependence of the ratio of metabolite fractional concentration changes to energy demand changes [$(dM/M)/dS$] on energy demand is plotted in Fig. 4. One can easily see that only ADP experiences significant relative concentration changes in most of the regulatory region. Relative changes in [ATP] are significant only at the onset of state 3, while relative changes in [P_i] are at best small and perceptible also only in the vicinity of the onset of state 3.

Fig. 5 presents simulated sensitivities of the respiration rate to metabolite concentrations [$\partial T/(\partial M/M)$] at different levels of energy demand. The oxygen consumption flux is significantly sensitive to relative changes in all three

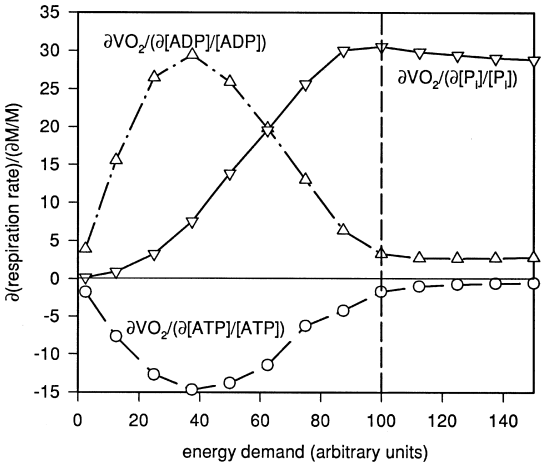


Fig. 5. Sensitivities of the respiration rate to relative changes in ATP, ADP and P_i $[\partial T/(\partial M/M)]$. The dashed line indicates the upper limit of the regulatory region.

metabolite concentrations. Therefore ATP, ADP, equally well as P_i, could potentially be effective regulators of respiration.

By comparison of Figs. 3–5 (the curves in Fig. 3 are products of the curves in Figs. 4 and 5), it is possible to conclude why ADP is the main intermediate of signal transduction from energy demand to the respiration rate and why its regulatory power is similar in most of the regulatory range of energy demand. The greatest fraction of mitochondria activation by an increased energy demand is transduced via ADP, because it is the only metabolite which exhibits a significant value of both $[(dM/M)/dS]$ and $[\partial T(\partial M/M)]$ in most of the regulatory range. The respiration rate is also sensitive to [ATP] and $[P_i]$, but relative changes in these metabolite concentrations are small during state 4 → state 3 transition. $(dT/dS)_M$ transduced by ADP is similar at different energy demands because, although $[(dM/M)/dS]$ and $[\partial T(\partial M/M)]$ of this metabolite change between different energy demand levels, the product of these parameters remains approximately constant (both parameter values change in the opposite directions).

Summing up, ADP is practically the only metabolite which regulates the rate of oxidative phosphorylation in response to varying energy demand in isolated mitochondria suspension. In other words, changes in this metabolite concentration are responsible for the main fraction of the integrated response of the respiration rate (target T) to energy demand (signal S) during the transition from state 4 to state 3. ATP and P_i play only a minor role at near-saturating energy demands. Moreover, the regulatory power of ADP, defined as a change in [ADP] multiplied by mitochondria sensitivity to [ADP] in a given state, has a similar value at most energy demand values in the regulatory region. This makes ADP an efficient and universal regulatory factor, increasing the respiration rate in response to an increased energy demand in isolated mitochondria.

However, it must be stressed that the above considerations concern only the regulation of ATP production in isolated mitochondria. In vivo (especially in intact muscle) the negative feedback via ADP concentration is probably only a secondary mechanism responsible for the adjustment of ATP supply to ATP demand, while the parallel activation of ATP production and ATP consumption by an external effector constitutes the main mechanism [20].

Acknowledgements

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