ELSEVIER

Biochimica et Biophysica Acta 1504 (2001) 31–45

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

Review

Theoretical studies on the regulation of oxidative phosphorylation in intact tissues

Bernard Korzeniewski *

Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, 31-120 Kraków, Poland

Received 1 May 2000; received in revised form 22 September 2000; accepted 29 September 2000

Abstract

The theoretical studies on the regulation of oxidative phosphorylation that were performed with the aid of kinetic models of this process are overviewed. A definition of the regulation of the flux through a metabolic pathway is proposed and opposed to the control exerted by particular enzymes over this flux. Different kinetic models of oxidative phosphorylation proposed in the literature are presented, of which only the model proposed by myself and co-workers was extensively used in theoretical studies on the regulation and compensation in the oxidative phosphorylation system. These theoretical studies have led to the following conclusions: (1) in isolated mitochondria, an increase in the activity of an artificial ATP-using system stimulates mitochondria mainly via changes in [ADP], while changes in [ATP] and [P_i] play only a minor role; (2) in non-excitable tissues (e.g. liver), hormones (acting via some cytosolic factor(s)) activate directly both ATP usage and at least some enzymes of the ATP-producing block; (3) in excitable tissues (e.g. skeletal muscle), neural signals stimulate (via some cytosolic factor(s)) in parallel all the steps of oxidative phosphorylation together with ATP usage and substrate dehydrogenation; (4) the decrease in the flux through cytochrome oxidase caused by a decrease in oxygen concentration is, at least partially, compensated by a decrease in Δp and increase in the reduction level of cytochrome *c*. A theoretical prediction is formulated that there should exist and be observable a universal cytosolic factor/regulatory mechanism which directly activates (at least in excitable tissues) all complexes of oxidative phosphorylation during an increased energy demand.

Keywords: Regulation of metabolism; Oxidative phosphorylation; Mitochondrion; Energy demand; Computer model; Theoretical prediction

1. Introduction

Oxidative phosphorylation in mitochondria is the main process responsible for the production of energy in the form of ATP in most animal tissues under most conditions; the general scheme of this process is presented in Fig. 1. The hydrolysis of ATP drives such processes in the cell as protein synthesis, DNA/RNA synthesis, ion (Na^+, K^+, Ca^{2+}) transport through the cell membrane, gluconeogenesis, ureagenesis, relative movement of myosin and actin filaments during muscle contraction and so on [1]. Nevertheless, the ATP turnover is not constant in time. During transition from resting state to active state, the energy demand increases up to two times in such non-excitable tissues as liver [2], and as much as tens or hundreds of times in skeletal muscle [3–9], which belongs to excitable tissues. Therefore, the

* Fax: +48-12-633-69-07; E-mail: benio@mol.uj.edu.pl

0005-2728/01/$ – see front matter
PII: S0005-2728(00)00237-1

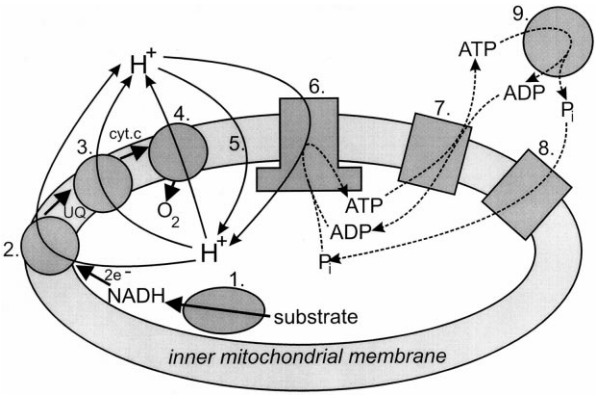

Fig. 1. Scheme of the oxidative phosphorylation system. 1., substrate dehydrogenation; 2., complex I; 3., complex III; 4., complex IV; 5., proton leak; 6., ATP synthase; 7., ATP/ADP carrier; 8., phosphate carrier; 9., ATP usage.

process of oxidative phosphorylation must be 'informed' in some way about the current energy demand, in order to meet the current ATP consumption.

It has been studied for decades how the ATP supply by oxidative phosphorylation in intact cells is regulated in response to a varying energy demand. It is well known that the external signals stimulating metabolism (and therefore the rate of mitochondrial respiration) are mostly different hormones (e.g. adrenaline, vasopressin, glucagon) in non-excitable tissues (liver, kidney) and neural stimulation in excitable tissues (skeletal muscle, heart, brain). However, the question remains as to the mechanism of intracellular regulation of ATP production and consumption. The first approach to this problem consisted in taking up studies on the regulation of respiration in isolated mitochondria. Chance and Williams [10,11] were the first to report that the oxygen consumption flux was stimulated in isolated mitochondria by an addition of a certain amount of ADP to the suspension of non-phosphorylating mitochondria. Thus, the so-called state 3 was reached, with maximal phosphorylation rate and oxygen consumption flux. When virtually all ADP molecules were transformed into ATP molecules, mitochondria passed to the so-called state 4, where the entire low oxygen consumption was due to proton leak [12]. It was subsequently demonstrated that the transition from state 4 to state 3, as well as to intermediate states, can be achieved by adding different amounts of an artificial ATP-consuming system (e.g. hexokinase, in the presence of glucose and ATP) [13] to a suspension of mitochondria. In this case, only ATP usage is activated directly (by an increase of the concentration of hexokinase), while particular mitochondrial complexes are activated indirectly, via changes in [ADP] and other intermediate metabolite concentrations. It had become widely accepted that the isolated mitochondria system resembles the situation prevailing in intact tissues. For example, it has been evidenced that – in muscle – the known intracellular signal (Ca^{2+} ions) released by neural stimulation activates directly ATP consumption (mainly actinomyosin-ATPase and Ca^{2+}-ATPase). Consequently, it was proposed that only ATP usage is directly stimulated in this case, and that mitochondria in resting skeletal muscle work in state 4, while mitochondria in maximally active skeletal muscle work in state 3, activated exclusively by an elevated concentration of ADP (and inorganic phosphate) [14].

The discovery of the activation in vitro of the irreversible TCA cycle dehydrogenases (pyruvate dehydrogenase, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase) by calcium ions prompted several authors [15–18] to postulate that at least some steps in the substrate dehydrogenation block (comprising TCA cycle, glycolysis and fatty acids β-oxidation), donating electrons (in the form of NADH and $FADH_2$) on the respiratory chain, are directly activated as well. On the other hand, the measured stimulation of isolated mitochondria by calcium ions was transient, weak, or none at all [19]. Therefore, the question concerning the physiological relevance and/or quantitative significance of the observed effect of Ca^{2+} on TCA cycle dehydrogenases remains open.

The above-mentioned proposals were mostly based on the studies on isolated mitochondria and/or enzymes. They also represented a qualitative (or, at best, semiquantitative) approach, since they offered only a qualitative explanation of what happens in intact tissues. On the other hand, when quantitative properties of (particular enzymes of) mitochondria were taken into account and confronted with the changes in the respiration rate and concentration of ADP (and also in other metabolite concentrations) taking place in intact cells during resting state → active state transition [20–22], it appeared that the di-

rect activation of either exclusively ATP usage or both ATP usage and substrate dehydrogenation cannot account for the situation occurring in vivo. Only a direct activation by some external cytosolic factor(s) of at least some (non-excitable tissues) [20] or all (excitable tissues) [21,22] enzymes of oxidative phosphorylation was able to explain the changes in fluxes and metabolite concentrations observed in intact tissues during activation of metabolism. This quantitative treatment was performed by means of one of the dynamic computer models of oxidative phosphorylation presented in the present article, namely the model developed by myself and co-workers [23–27]. The discussed theoretical studies had led to the each-step activation proposal, intended to reflect the mechanism of regulation of oxidative phosphorylation in skeletal muscle during transition from resting state to intensive exercise [21,22].

Apart from a varying energy demand, another disturbance of the oxidative phosphorylation system which is of a physiological relevance consists in a decrease of the oxygen concentration to very low levels (hypoxia), occurring during an intensive prolonged skeletal muscle exercise or in some pathological states of heart (ischaemia). The apparent K_m constant of oxidative phosphorylation for $[O_2]$ is very low in intact tissues, usually smaller than 1 μM [28]. Significant changes in intermediate metabolite concentrations (cytochrome *c* reduction level, phosphorylation potential) begin, however, at much higher oxygen concentrations, greater than 10 μM [29–31]. This suggests that it is an appropriate metabolic compensation that enables the existence of so high an affinity of oxidative phosphorylation as a whole to the oxygen concentration. Indeed, computer modelling of aerobiosis → anaerobiosis transition in isolated mitochondria and intact cells [23,26] showed that changes in the protonmotive force and cyt.c^{2+}/cyt.c^{3+} ratio counter-act the decrease in oxygen pressure and thus help to keep the flux through cytochrome oxidase as constant as possible. Computer simulation predicted also that, at low oxygen concentrations, cytochrome oxidase takes over a great part of the control over the oxygen consumption flux from other steps of oxidative phosphorylation [32], which was later confirmed by experimental studies [33]. Therefore, the use of computer modelling of oxidative phosphorylation has led to a formulation of new proposals concerning the regulation of this process in intact tissues both during an increased energy demand and at a significantly lowered oxygen tension.

The present article is intended to show that well-tested dynamic computer models of biochemical pathways (in this case: oxidative phosphorylation) are potentially a very useful and powerful tool for studying and understanding the regulation of metabolism, and allow a deeper insight into the mechanisms underlying this regulation. They are also able to predict new phenomena, the existence of which can be later verified (or falsified) in the experimental way.

2. Regulation vs. control vs. compensation

To talk about the regulation of oxidative phosphorylation, one must first define strictly the notion 'regulation'. The terms 'control of flux' and 'regulation of flux' are frequently confused, or even used as synonyms. In the present article, I would like to distinguish clearly between control and regulation. The control of flux has been defined strictly in the frame of metabolic control analysis (MCA) [34–36]. The extent to which particular enzymes control the flux through a given metabolic pathway has been expressed quantitatively in the form of flux control coefficients. The flux control coefficient is defined as the ratio of a relative change in the flux J through the metabolic pathway m caused by a small (infinitesimal) change in the concentration (activity) of the enzyme E_i to this change itself:

$$C_{E_i}^{J_m} = (dJ_m/J_m)/(dE_i/E_i). \quad (1)$$

Therefore, the control of flux determines only a potential ability of changing the flux when the concentration or activity of a given enzyme is changed by some external effector. On the other hand, the control of flux says nothing about which enzyme activities are really changed under physiological conditions. The response of particular enzyme activities to external effectors is described within MCA by the elasticity coefficients to an external effector, while the response of the overall flux through a considered system – by the response coefficient to an external

effector [34]. Nevertheless, the coefficients of MCA concern only infinitesimal changes in the system and therefore they cannot describe properly a transition between two different steady-states, where large changes in fluxes and metabolite concentrations can take place.

I would like to define the regulation of flux as the physiological mechanism(s) responsible for a transition (involving large-scale changes in fluxes and metabolite concentrations) of a given metabolic system from one steady-state to another steady-state, caused by some purposeful (in the physiological sense) signal from outside of the system. In particular, the regulation of flux refers to the mechanism determining which enzymes are directly activated by an external effector and to what extent. For infinitesimal changes, this is equivalent to the fact which enzymes have non-zero response coefficients to a given external effector. Generally, two kinds of regulation can be distinguished: direct regulation and metabolite-mediated regulation. In the case of direct regulation (described within MCA by response coefficients to an external effector), the flux through a given metabolic block is increased (decreased) by a direct activation (inhibition) by an external effector of this block in general, and of its different enzymes in particular. In the case of metabolite-mediated regulation (expressed within MCA as internal response to intermediate metabolite concentrations), a metabolic block in question is not directly activated: an external effector stimulates some enzymes situated upstream and/or downstream of this block, while the flux through the block increases due to an increase in the concentration of its substrates and/or decrease in the concentration of its products. Therefore, this kind of regulation must involve significant changes in metabolite (substrate and/or product) concentrations, unless all enzymes of the block are extremely sensitive to intermediate metabolite concentrations and have a sufficiently great capacity for an increase in the flux. Generally, the two distinguished kinds of regulation can be formulated (at least for infinitesimal changes) within MCA in terms of internal responses (to intermediate metabolites) and external responses (to external effectors).

I would also like to distinguish between regulation and compensation. While regulation involves stimulation of a given metabolic system by purposeful (from the physiological point of view) external signals (e.g. hormones, neural stimulation), compensation refers to the situation when there takes place an undesired shortage of some – delivered from outside (e.g. by the circulation of blood) – substrate of a considered biochemical pathway. Such shortage causes an unfavourable decrease in the flux that can be (at least partially) compensated by appropriate changes in intermediate metabolite concentrations. Thus, the decrease in the flux can be minimised. The decrease in the flux would be much greater without such a compensation (when the concentrations of relevant intermediate metabolites were kept constant). Therefore, compensation leads to an internal homeostasis of the cell.

In the context of the above-defined regulation of flux, three questions should be answered to determine how a given metabolic block is regulated in response to some signal from outside: (1) which enzymes (if any) of this block, as well as which enzymes upstream and downstream of this block, are directly activated by an external effector, and to what extent; (2) what is a quantitative contribution of the direct regulation and metabolite-mediated regulation to the increase in the flux; (3) what is the quantitative contribution of different metabolites to the metabolite-mediated regulation.

As to the compensation for a decrease in a flux caused by some external substrate shortage, one should know: (1) what fraction of the decrease in the flux is compensated by changes in intermediate metabolite concentrations; (2) what is the quantitative contribution of particular intermediate metabolites to this compensation.

Referring the above formulation of the regulation of flux to the oxidative phosphorylation system allows the problems concerning the regulation of this system to be re-formulated as follows. The oxygen consumption (and oxidative ATP synthesis) flux in particular tissues and cells is increased by some signal (S) from outside (hormone, neural stimulation) in the physiological conditions of an intact organism. This signal is transmitted via some intracellular factor (X), which is an external effector (and not an intermediate metabolite) for some enzyme(s) (block(s)) of the bioenergetic system of a cell. The mechanisms of the regulation of oxidative phosphorylation proposed in the literature can be re-

named and characterised in the discussed context as follows.

(A) Output activation. Only ATP usage (output of the system), situated downstream of oxidative phosphorylation, is directly activated by X (at least in some cases: calcium ions); particular mitochondrial enzymes are activated indirectly by changes in [ADP] and other metabolite concentrations (e.g. particular complexes of the respiratory chain are activated by a decrease in Δp); similarly, the substrate dehydrogenation block (including TCA cycle, glycolysis, fatty acids β-oxidation, glucose and fatty acids transport) is activated indirectly, by an increase in [ADP], [NAD^+] and possibly other metabolite concentrations. This mechanism is an example of the metabolite-mediated regulation (if the considered metabolic block is oxidative phosphorylation). The output activation mechanism is presented schematically in Fig. 2A.

(B) Input/output activation. Only ATP usage (output) and substrate dehydrogenation (e.g. irreversible TCA cycle dehydrogenases: pyruvate dehydrogenase, isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase) (input of the system, situated upstream of oxidative phosphorylation) are directly activated by X (e.g. calcium ions), while mitochondria are activated indirectly, by an increase in the concentration of ADP and/or NADH. The input/output activation mechanism, being also an example of the metabolite-mediated regulation (in relation to oxidative phosphorylation), is presented in Fig. 2B.

(C) Each-step activation. The external effector X activates directly ATP usage, substrate dehydrogenation as well as all steps of the oxidative phosphorylation system. In the idealised case where all enzymes are activated to exactly the same extent, and therefore no changes in intermediate metabolite concentrations take place, the each-step activation mechanism is a pure example of the direct regulation. In a more realistic case, where different steps are directly activated to a slightly different extent and therefore some small variations in metabolite concentrations occur, the each-step activation mechanism is a mixture of the direct regulation and metabolite-mediated regulation, yet the direct regulation predominates as a mechanism increasing the flux. The each-step activation mechanism is presented in Fig. 2C.

The kinetic modelling discussed in the present article helps to answer the following questions: (1) which of the mechanisms of the regulation of oxidative phosphorylation proposed in the relevant literature (output activation, input/output activation or each-step activation) occurs in intact tissues; (2) what is the relative contribution of the direct regulation and metabolite-mediated regulation to the stimulation of respiration (and ATP synthesis) flux during resting state → active state transition; (3) what is the relative contribution of the changes in different

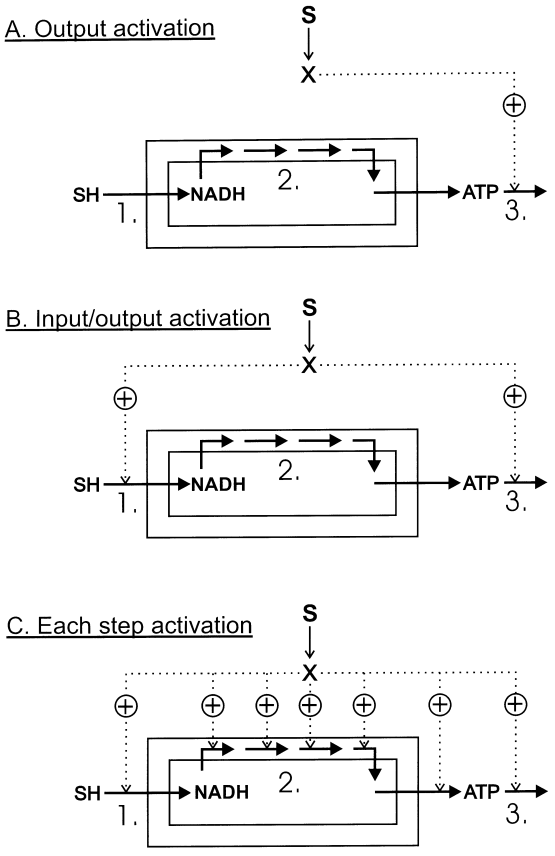

Fig. 2. Three possible mechanisms of regulation of oxidative phosphorylation. Particular steps are either activated directly, by an external effector (dotted arrows), or only indirectly, via changes in intermediate metabolite concentrations. S – signal from outside (hormone, neural signal); X – intracellular effector (external in relation to oxidative phosphorylation); SH – respiratory substrate; 1., substrate dehydrogenation; 2., particular steps of oxidative phosphorylation; 3., ATP usage.

metabolite concentrations (e.g. [ATP], [ADP] and [P_i]) to the metabolite-mediated regulation in intact tissues and, especially, in isolated mitochondria.

As to the compensation for a decrease in a flux caused by a substrate shortage, oxidative phosphorylation can sometimes work in the conditions where oxygen or respiratory fuels are maintained at low levels in blood. For example, hypoxia can occur in skeletal muscle during prolonged intensive exercise. The relevant questions in this context are: (1) what fraction of the decrease in the oxygen consumption flux caused by a decreased oxygen concentration in blood is compensated by changes in intermediate metabolite concentrations (in the case of whole mitochondria: e.g. in the ATP/ADP ratio and NADH/NAD^+ ratio); (2) what is the relative quantitative contribution of different metabolites to this compensation. The present paper discusses theoretical studies on the compensation for a decrease in the flux through cytochrome oxidase at low oxygen concentrations by changes in the protonmotive force and reduction level of cytochrome *c*, performed with an aid of a kinetic model of oxidative phosphorylation.

3. Kinetic models of oxidative phosphorylation

The major and fundamental question concerning kinetic modelling is: why to model metabolic pathways? What profits can be provided by computer models that cannot be offered by a purely experimental approach? A model is an approximate representation of some aspects of reality selected by the author(s) of a given model. Each model is usually a compromise between oversimplification and excessive sophistication. A kinetic computer model of a metabolic pathway (e.g. oxidative phosphorylation) constitutes a quantitative mathematical description of selected elements and parameters of the modelled system, chiefly enzymatic reaction rates and metabolite concentrations. Of course, any model cannot be anything more, but a simplified representation of the biochemical pathway it refers to, because such a model is based on many assumptions and approximations. Additionally, one can never be sure that all important elements of the modelled system have been discovered experimentally, and thus, that they are covered by the model. Therefore, each model has only a limited area of its applicability, and theoretical predictions performed with the aid of a model should be treated with caution and ultimately verified (or falsified) in the experimental way. It is extremely important for any model of a complex metabolic pathway to be tested for a possibly broad set of parameter values, conditions and different properties of the modelled system. Each successful test increases the reliability of a model. Models usually contain free parameters, the values of which must be adjusted. Therefore, it is relatively easy to adjust many different models to the same simple set of parameter values and system properties. For this reason, a thorough and extensive verification of a model is of great importance.

On the other hand, well-tested kinetic models can prove to be very useful research tools. Such models help to interpret, analyse and process a great number of quantitative and semiquantitative experimental data concerning a sophisticated metabolic system under consideration. Computer modelling forces a researcher to formulate all the problems in an explicit and quantitative way, which takes place only infrequently in the case of intuitive considerations. Kinetic models allow one to investigate how the macroscopic level of the cell behaviour emerges from the interplay of particular elements on the microscopic level of enzymatic reactions. They also help to check in a quantitative way if all known elements and their properties suffice to explain the entire behaviour of the investigated metabolic system in vivo, or else, if some new factors possessing properties determined by a model should be looked for. Human brain could not cope by itself with such a sophisticated, multi-factor analysis.

Kinetic models can be generally divided into static models and dynamic models. In contrast to static models, dynamic models involve changes of variable values (reaction rates, metabolite concentrations) in time. Within a dynamic kinetic model, the rate of each kinetic reaction, block of reactions and process is described by an appropriate kinetic equation, expressing the dependence of this rate on different metabolite concentrations. Variations in time of different metabolite concentrations are expressed as sets of differential equations; in such an equation, the resultant rate is the difference between the sum of the rates of reactions producing a given metabolite and

the sum of the rates of reactions consuming this metabolite. In subsequent steps (representing time intervals), new values of rates and concentrations in a new moment in time are calculated on the basis of the old values of rates and concentrations at the previous moment in time. The size of steps (time intervals) must be small enough to ensure the stability and accuracy of the solution; an excessively small step, however, would increase unnecessarily the time of a simulation. Therefore, the step size is usually adjusted automatically by appropriate integration procedures, that also optimise the rate of calculations. On the other hand, static kinetic models allow only to calculate steady-state values of fluxes and some metabolite concentrations, but cannot simulate their changes in time.

One can arbitrarily distinguish four stages in the use of a kinetic model of a metabolic pathway. In the first stage, a newly developed model must be extensively tested, i.e. compared to a broad set of various experimental data and, if necessary, modified in order to obtain a good (semi)quantitative agreement between computer simulations and experiments. Next, the explicitly formulated description of particular elements of the modelled system (required in the process of model building) can be used for a better understanding, interpretation and insight into the function of this system. Thirdly, the model can be used to calculate variable values and to simulate different dynamic properties that have not yet been measured experimentally (e.g. flux control coefficients of particular enzymes of oxidative phosphorylation in muscle mitochondria in state 3.5 and at low oxygen concentrations [27,32]). The fourth, most ambitious stage consists in attempting to predict the existence of completely new phenomena that have not been discovered yet in the experimental way (e.g. a universal external activator of all complexes of oxidative phosphorylation in muscle [21,22]). Of course, all such predictions have to be verified (or falsified) by experimental studies. Nevertheless, theoretical predictions obtained with an aid of a computer kinetic model can inspire and direct further experimental studies.

Several kinetic models of oxidative phosphorylation, both static and dynamic, have been proposed in the relevant literature. The first, very simple kinetic description of oxidative phosphorylation in isolated mitochondria was formulated by Chance and Williams [10,11], where the dependence of the respiration rate on ADP concentration was described by the Michaelis–Menten kinetics, with a K_m value of about 25 μM. This approximate, phenomenological, black-box approach is still frequently used, because it works well enough in many cases where the detailed knowledge about what happens inside isolated mitochondria is not needed.

Wilson, Ereciñska and colleagues developed a more sophisticated static model of oxidative phosphorylation [30,31]. They assumed that the only rate-limiting step in this process was cytochrome oxidase, while other steps were close to thermodynamic equilibrium and therefore did not control the flux. For this reason, this model was in fact a model of cytochrome oxidase. The kinetic expression for the rate of the reaction catalysed by this enzyme included the dependence on the external ATP/ADP*P_i ratio, oxygen concentration and the reduction level of cytochrome c: cyt.c^{2+}/cyt.c^{3+} (and, indirectly, via thermodynamic equilibrium of the respiratory chain, on the [NADH]/[NAD^+] ratio). This model was afterwards transformed into a dynamic model by Korzeniewski and Froncisz [37].

The Wilson's model in its original (static) version was able to predict correctly (at least in a semiquantitative way) the value of the respiration rate at different combinations of the external phosphorylation potential, cyt.c^{2+}/cyt.c^{3+} and NADH/NAD^+ ratio [30,31]. It also described properly the experimentally measured dependence of the respiration rate, external ATP/ADP*P_i ratio, NADH/NAD^+ ratio and reduction level of cytochrome c on the oxygen concentration [30]. Simulations performed with an aid of the dynamic version of the discussed model [37] agreed well with the experimentally observed time courses of [O_2] and cyt.c^{2+}/cyt.c^{3+} during oxygen consumption by a suspension of isolated mitochondria in a closed chamber, leading finally to a complete exhaustion of oxygen [30].

From the point of view of the present knowledge, the Wilson's model seems to be outdated. The assumption of one rate-limiting step is completely invalid from the point of view of MCA [34–36]; on the contrary, this approach has clearly demonstrated that the control is shared more or less uniformly by virtually all the complexes of oxidative phosphor-

ylation [13,38]. Additionally, the discussed model does not take into account the Mitchell's chemiosmotic theory [39] and involves a dependence of cytochrome oxidase on the external phosphorylation potential rather than on the protonmotive force (Δp). Taking into account these facts, one must admit that Wilson's model yields a surprisingly good agreement with some experimental results. This observation exemplifies the above-discussed statement that many different models can be adjusted (by fixing appropriate free parameter values) to the same small set of experimental data.

Oxidative phosphorylation was also modelled by means of the thermodynamic, and not kinetic, approach, within the framework of the so-called non-equilibrium thermodynamics (NET) [40,41]. Within this approach, fluxes are described as functions of thermodynamic forces. The thermodynamic approach successfully describes some bioenergetic properties of isolated mitochondria [42,43]. However, it is based on several assumptions that are invalid in many conditions (e.g. assumptions concerning the linearity of the flux–force relationship or unique dependence between the flux and the force [26]) and represents a phenomenological, black-box description (NET distinguishes only two fluxes and two forces in the oxidative phosphorylation system). Additionally, NET assumes that fluxes are only regulated by thermodynamic forces and therefore implies implicitly the metabolite-mediated type of regulation. Thus, the approach assumes what should be first proven to take place in intact tissues. For this reason, NET will not be discussed more extensively in the present article.

The static kinetic model of oxidative phosphorylation developed by Bohnensack [44–46] was very important for at least three reasons. It was the first one to involve explicitly most of the complexes of oxidative phosphorylation in mitochondria presented in Fig. 1 (though it treated the whole respiratory chain as one entity and assumed that the phosphate carrier worked in thermodynamic equilibrium). It took into account the chemiosmotic theory and therefore involved the proton (and charge) gradient through the inner mitochondrial membrane. Finally, it was consistent with the fact that the control of the flux is shared by many steps of oxidative phosphorylation.

The Bonensack's model was able to predict at least semiquantitatively the dependence of the respiration on the external ATP/ADP ratio and the pattern of metabolic control in a few sets of conditions [44–46]. However, it has not been tested for a broader set of experimental data. Moreover, some kinetic equations contained in this model seem to be too simplified. Finally, this model, as a static one, is not able to simulate time courses of different parameter values (especially metabolite concentrations) during transition from one steady-state to another steady-state.

The first dynamic model of oxidative phosphorylation was developed by Holzhütter and co-workers [47]. This model, however, was tested for a rather limited set of conditions. What is more, one can hardly agree with some assumptions made by the authors, for instance those concerning the three enigmatic proton pumps before cytochrome oxidase, linear dependence of the proton leak on Δp or the form of kinetic equations B and D in table I in [47]. It is also noticeable that the size of the internal adenine nucleotide pool was underestimated in order to obtain time courses of parameters consistent with experiments. Finally, the authors developed their model to simulate experiments performed in an unphysiologically low temperature (8°C).

The dynamic kinetic model of oxidative phosphorylation – developed by myself and co-workers in three versions: for liver mitochondria [23,24,26], intact hepatocytes [20,25] and skeletal muscle mitochondria [27] – is in its general structure quite similar to Bohnensack's and Holzhütter's models. It takes into account explicitly all the complexes of oxidative phosphorylation presented in Fig. 1 as well as some additional phenomena (e.g. magnesium complexes of adenine nucleotides). Generally, it possesses all the properties of a dynamic kinetic computer model presented above. The most important difference (leaving aside the fact that, for instance, Bohnensack's model is a static one) is that particular kinetic equations within this model, initially assumed on the basis of the incomplete kinetic data available in the literature, were appropriately modified later, in order to fit the existing experimental results, and that the whole model was extensively tested for a possibly broad set of variable values and system properties. The discussed model was able to represent in a (semi)quan-

titative way the following properties and parameter values of oxidative phosphorylation:

- changes in different variable values (respiration rate, Δp, reduction level of cytochrome *c*, internal and external ATP/ADP ratio and others) during state 4 → state 3 transition in isolated mitochondria [24];
- time courses of these variables after an addition of a small amount of ADP to mitochondria in state 4 (state 4 → state 3 → state 4 transition) [24];
- flux control coefficients for different components of oxidative phosphorylation with respect to the respiration flux at different respiration rates between state 4 and state 3 [24,27], obtained within the framework of MCA [13,38];
- flux control coefficients for the oxidation, phosphorylation and proton leak subsystems over the oxidation (respiration), phosphorylation and proton leak fluxes at different respiration rates between state 4 and state 3 [24], obtained within the framework of the 'top-down approach' to MCA [48];
- time courses of different variable values during consumption of oxygen by suspension of cells (or mitochondria) in a closed chamber (aerobiosis → anaerobiosis transition) [23,26];
- kinetic responses of oxidation, phosphorylation and proton leak subsystems to Δp in hepatocytes incubated with different respiratory substrates [25];
- values of the respiration rate, cytochrome *c* reduction level and external ATP/ADP ratio at different oxygen concentrations [26];
- linear (at least in some range) flux–force relationships, similar to those obtained for oxidative phosphorylation within the framework of NET [26];
- inhibitor titration curves for particular complexes of oxidative phosphorylation in skeletal muscle mitochondria [27], obtained experimentally in the frame of MCA [38].

The agreement between simulations and experiments was at least semiquantitative, and in many cases very good. On this basis, the model was subsequently used in several theoretical studies on the control and regulation of oxidative phosphorylation in isolated mitochondria and intact cells and tissues [20–22,26,27,32,49]. Mainly these theoretical results are discussed in the present article.

Aliev and Saks proposed a dynamic kinetic model of oxidative phosphorylation in intact heart [50], including creatine kinase (CK) and the phosphocreatine (PCr)/creatine (Cr) pair. They emphasised possible limitations of the diffusion of adenine nucleotides (especially ADP) and displacement of CK from equilibrium, while the 'mitochondrial' part of the model was very simplified. The authors developed their model mainly in order to explain small changes in [ADP] accompanying large variations in the respiration and ATP synthesis fluxes in heart in situ [51,52]. The results of their simulations, however, contradict in several cases experimental data (see below). Therefore, the Aliev and Saks' model – although it constitutes an interesting attempt to explain the behaviour of the bioenergetic system in heart – seems to need some modifications before it can be accepted.

4. Response to an increased energy demand

4.1. Intact cells and tissues

The model developed by myself and co-workers [23–27] was the only one to be used extensively to study regulation and compensation, as defined above, in the case of oxidative phosphorylation system in intact cells and tissues [20–22,26,27,32,49]. The regulation of ATP supply at a varying energy demand involves the question of which steps (if any) of oxidative phosphorylation are directly activated by an intracellular effector transmitting some signal from outside, and to what extent. To answer this, one must refer to the changes in fluxes and metabolite concentrations, that take place during transition from resting steady-state to active steady-state. It is also necessary for such an analysis to account for the quantitative kinetic properties of particular steps of the activated system (in this case: oxidative phosphorylation), especially their capacities for an increase in the flux and sensitivities to intermediate metabolite concentrations. This is what a well-tested kinetic model can offer. Namely, such a model can help to determine which enzymes should be directly activated in order to explain the changes in fluxes

and metabolite concentrations observed experimentally.

When respiration and ATP turnover are activated in hepatocytes by hormones acting via Ca^{2+} ions (e.g. vasopressin), there takes place a small increase in some metabolite concentrations (NADH/NAD^+, cyt.c^{2+}/cyt.c^{3+}, mitochondrial ATP/ADP) [53,54], while ΔpH remains essentially constant [55]. The activation of hepatocytes by these hormones also leads to a decrease in the flux control coefficient of the ATP/ADP carrier [56]. The quantitative analysis performed with the aid of the version of the discussed model of oxidative phosphorylation prepared for hepatocytes [20] suggests that ATP usage, substrate dehydrogenation and at least some complexes of oxidative phosphorylation in mitochondria (e.g. ATP/ADP carrier) should be directly activated in order to explain the above experimental results. These theoretical predictions inspired experimental studies that at least partially confirmed them. Namely, it was shown – within the proportional activation approach – that vasopressin activates simultaneously and to a very similar extent at least the Δp-producing block and Δp-consuming block [57]. Subsequently, it was shown that adrenaline and glucagon affect directly (via some intracellular factor) most of the elements of the bioenergetic system in hepatocytes [58].

A huge increase (15–200 times) in the respiration rate takes place in mammalian skeletal muscle during transition (initiated by neural signals) from resting state to intensive exercise, accompanied by only a modest decrease (2–5 times) in the ATP/ADP ratio [3–8]. At the same time, the NADH/NAD^+ ratio can either increase or decrease [59–64]. Additionally, the capacity of oxidative phosphorylation for the oxygen consumption flux is about 2–5 times greater in intact muscle than in isolated mitochondria [65–67]. Computer simulations performed by means of the version of the model for muscle mitochondria [27] led to the conclusion that not only ATP usage and substrate dehydrogenation, but also all mitochondrial complexes of oxidative phosphorylation should be directly activated to a large extent (about 10 times or so) in order to account for the above experimental results [21]. When only ATP usage or only ATP usage and substrate dehydrogenation were directly activated in simulations, a much smaller than expected increase in the respiration rate was obtained, while the ATP/ADP ratio dropped dramatically below 1. Therefore, the output activation (Fig. 2A) and input/output activation (Fig. 2B) mechanisms failed completely as a quantitative explanation of the above-mentioned experimental data. A quite similar behaviour of the system was observed in computer simulations, even when only one complex of oxidative phosphorylation was not directly stimulated. It was only a direct activation of all steps of oxidative phosphorylation (each-step activation mechanism, Fig. 2C) that yielded the expected changes in the respiration rate and ATP/ADP ratio [21]. Additionally, it was demonstrated that the direct regulation predominates over the metabolite-mediated activation during resting state → intensive exercise transition in skeletal muscle: about 80% of the increase in the respiration rate during muscle stimulation was due to a direct activation of oxidative phosphorylation, while the remaining 20% was due to an increase in the ADP concentration [21]. Therefore, the studies based on the theoretical model decidedly favour the each-step activation mechanism of the regulation of oxidative phosphorylation in skeletal muscle in response to a largely increased energy demand. The problem of regulation of oxidative phosphorylation in skeletal muscle is discussed broadly in [22].

In heart in situ, essentially no changes in the ATP/ADP ratio and NADH/NAD^+ ratio take place during large variations in the flux [51,52,68]. Therefore, mitochondria can be activated significantly neither by an increase in the later ratio (input activation), nor by a decrease in the former ratio (output activation). For this reason, the metabolite-mediated regulation does not seem to play an important role in the regulation of oxidative phosphorylation in heart in vivo. This strongly supports the conclusion that at least some, and possibly all complexes of oxidative phosphorylation are directly activated during an increase in heart beating frequency. Indeed, it was proposed previously that ATP synthase in heart is stimulated by calcium ions [69]. Nevertheless, Aliev and Saks proposed – according to their model [50] – that the existing experimental data can be explained exclusively by the compartmentalised energy transfer, without the necessity to refer to the direct activation of oxidative phosphorylation, or even of substrate dehydrogenation. Unfortunately, their simulations remain in contradiction with several experimental re-

sults. The authors' simulations suggest explicitly that the PCr/ATP ratio decreases two times during a 5-fold increase in the flux (figure 14B in [50]), while essentially no changes in this ratio were observed in experiments [51,70]. In fact, their general explanation has little to do with the postulated compartmentalised energy transfer, and is based on the assumption that P_i concentration is very low (a few μM) in a slowly beating heart, and therefore it limits the flux, while this concentration increases dramatically (to 12 000 μM, by 3–4 orders of magnitude) during activation of heart work (figure 14D in [50]). This assumption stands in severe conflict with many experimental results, where relatively small (by about 50–100%) or no changes in $[P_i]$ are reported at all [70–73], while the (measured or estimated) inorganic phosphate concentration in slowly beating heart is, roughly, between 500 and 2000 μM [70–75]. Also the extended version of this model [76] predicts huge relative changes in $[P_i]$ (see figure 9 in [76]). Additionally, the Aliev and Saks' proposal cannot explain the relatively constant $NADH/NAD^+$ ratio in heart [68]. Therefore, it seems that a parallel direct activation of several (possibly all) steps of oxidative phosphorylation is necessary for the explanation of the behaviour of the bioenergetic system in intact heart. In this case as well, the each-step activation mechanism seems to fit best the existing experimental data.

Summing up, computer modelling of the regulation of oxidative phosphorylation in response to increased energy demand in intact tissues leads to the conclusion that the direct regulation predominates in this case over the metabolite-mediated regulation (at least in excitable tissues), and that the each-step activation mechanism describes best the situation taking place in vivo. Therefore, one can postulate the existence of a universal activator of all steps of oxidative phosphorylation of a still unknown nature. I proposed previously [22] that the factor can be found in the frequency of calcium oscillations (in the case of hepatocytes: the so-called calcium spiking) generated in cells in response to external stimuli (hormones or neural signals), and that this frequency can be integrated over time by some protein which causes e.g. a phosphorylation of oxidative phosphorylation complexes. This supposition should, of course, be tested experimentally.

4.2. Isolated mitochondria

In isolated mitochondria, during transition from state 4 to state 3 induced by an increasing activity of an artificial ATP-usage system (e.g. increasing amount of hexokinase in the presence of glucose), the only relevant 'regulatory mechanism' is of course output activation (Fig. 2A). The rate of respiration (and ATP synthesis) is activated exclusively indirectly, via an increase in [ADP] and $[P_i]$, and a decrease in [ATP] (metabolite-mediated regulation). Changes in all three concentrations could, at least potentially, affect the respiration rate. It was in fact observed experimentally that different, externally imposed, combinations of ATP, ADP and P_i concentrations yield different values of oxygen consumption by suspension of isolated mitochondria [77,78]. This prompted several authors to postulate that ADP concentration [10,11], ATP/ADP ratio [79,80], ATP/ADP*P_i ratio [81], phosphorylation potential [82,83] or Atkinson's energy charge [84] 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 determine the sensitivity of mitochondria to particular metabolites, but it was not possible to determine how the metabolite concentrations change as energy demand increases. The concentrations of ATP, ADP and P_i are not related randomly to each other during an increased energy demand; 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 satisfactorily in a quantitative way. Therefore, the question is which metabolites participate in the metabolite-mediated regulation of oxidative phosphorylation in isolated mitochondria during state 4 → state 3 transition, and to what extent they do so.

Computer simulations performed with an aid of the discussed model of oxidative phosphorylation suggest that ADP is the main regulatory factor which

stimulates respiration in isolated mitochondria in response to an increased energy demand, mostly because large relative changes in the concentration of this metabolite [49]. Changes in [ATP] and [P_i] contribute to the respiration rate increase very weakly, and only in the nearest vicinity of state 3. Generally, changes in [ADP] are responsible for approximately 90% of the respiration rate increase during state 4 → state 3 transition, while the remaining approximately 10% is due to changes in [P_i] and [ATP] [49]. Therefore, instead to postulate that oxidative phosphorylation in isolated mitochondria is regulated by e.g. ATP/ADP ratio or phosphorylation potential, it is better to say that most of the regulatory signal is transmitted by ADP, while the rest by ATP and inorganic phosphate.

As it was discussed above, in intact tissues, both metabolite-mediated regulation and direct regulation take part in the determination of the oxygen consumption flux (direct regulation predominates in excitable tissues at higher degrees of activation). Therefore, the discussed theoretical results suggest that ADP can be the main regulatory signal transmission route within the metabolite-mediated regulation. On the other hand, one should bear in mind that changes in [P_i] are greater in excitable tissues than in isolated mitochondria, because of the presence of CK and the PCr/Cr pair. Even in this case, however, ADP seems to be more important as a regulatory metabolite, since oxidative phosphorylation, for instance in skeletal muscle, is probably near-saturated with inorganic phosphate already in resting state [22].

5. Compensation of a decreased oxygen concentration

Oxygen is one of the substrates of oxidative phosphorylation, in particular of cytochrome oxidase. When the concentration of oxygen decreases, one should expect that the concentrations of other metabolites will change in order to counter-act the decrease in the respiration rate caused by oxygen shortage. In other words, other metabolites should compensate for the decrease in the oxygen consumption flux. The relevant 'metabolites' are the reduction level of cytochrome *c* (cyt.c^{2+}/cyt.c^{3+}) and protonmotive force (Δp) in the case of cytochrome oxidase, and the NADH/NAD^+ and ATP/ADP ratios in the case of oxidative phosphorylation in general. Indeed, Wilson and co-workers observed an increase in the reduction level of cytochrome *c* and NAD as well as a decrease in the cytosolic phosphorylation potential that accompanied a decrease in the oxygen concentration [29,30].

The above-discussed dynamic kinetic model of oxidative phosphorylation was used to study in a quantitative way the compensation for a decrease in the flux through cytochrome oxidase (resulting from a decrease in [O_2]) by an increase in the cyt.c^{2+}/cyt.c^{3+} ratio and a decrease in Δp [26]. Firstly, it was demonstrated that the decrease in the respiration at lowered oxygen concentrations would be about 10 times greater without this compensation, when the concentrations of these metabolites were kept constant. In other words, changes in the reduction level of cytochrome *c* and protonmotive compensate for about 90% of the decrease in the flux, brought about by a decrease in the oxygen concentration. This offers an attractive explanation of why the apparent (involving implicitly changes in cyt.c^{2+}/cyt.c^{3+} and Δp) Michaelis–Menten constant of oxidative phosphorylation for oxygen has a very low value (< 1 μM) and is probably much smaller than the 'real' Michaelis–Menten constant (defined for constant values of cyt.c^{2+}/cyt.c^{3+} and Δp).

Secondly, it was calculated that changes in the reduction level of cytochrome *c* and changes in the protonmotive force have a quantitatively similar contribution to the compensation for a decrease in the flux through cytochrome oxidase caused by a decrease in oxygen concentration [26]. Therefore, both factors are equally important in keeping the respiration rate as constant as possible. The situation is quite different in the case of state 4 → state 3 transition in response to an increased activity of an artificial ATP-usage system in isolated mitochondria: there, a decrease in Δp is the main factor responsible for stimulation of the flux through cytochrome oxidase, while the reduction level of cytochrome *c* plays only a minor role [26]. This exemplifies the fact that different metabolites can have a different regulatory significance in the same system, when this system is exposed to different changes in the external conditions.

Finally, computer simulations predicted that the

flux control coefficient of cytochrome oxidase increases significantly at very low oxygen concentrations (around the K_m constant of oxidative phosphorylation for oxygen) [32]: the metabolic control is taken over from other enzymes. This reflects the fact that the compensation capacity of the system is exhausted at extremely low levels of oxygen. These theoretical predictions were later confirmed experimentally [33]. Of course, the fact that the flux control coefficient of cytochrome oxidase increases at low oxygen concentrations can be expected even without computer modelling. Because oxygen is an external metabolite for oxidative phosphorylation, a decrease in its concentration lowers the effective activity of cytochrome oxidase, which should lead, in a typical case, to an increase in the flux control coefficient of this enzyme. Nevertheless, a computer model can predict what is a quantitative extent of this increase and at which oxygen concentrations it takes place.

In the future, it would be interesting to study (in a theoretical or experimental way) the compensation of a decrease in oxygen concentration by NADH/NAD^+ and ATP/ADP in the context of entire mitochondria.

A possible compensation of a respiratory substrate shortage has not been studied in a theoretical way. However, it seems probable that the decrease in the flux through the substrate dehydrogenation block caused by a diminished respiratory substrate concentration is compensated by a decrease in the NADH/NAD^+ ratio, while the decrease in the flux through oxidative phosphorylation by a decrease in the ATP/ADP ratio.

6. Conclusions

The present article is intended to show that a well-tested dynamic kinetic model of a metabolic pathway (in this case: oxidative phosphorylation) can be a very useful research tool, that helps to integrate and analyse a sophisticated set of quantitative experimental data. The simulations performed with the computer model developed by myself and co-workers have led to the following general conclusions concerning the regulation of oxidative phosphorylation in intact tissues: (1) the each-step activation mechanism – where all steps of oxidative phosphorylation are directly activated by some external effector (Fig. 2C) – seems to fit best the entire set of the existing experimental data; (2) both direct regulation and metabolite-mediated regulation contribute to the increase in the flux through the oxidative phosphorylation system during an elevated energy demand (direct regulation predominates at least in excitable tissues at large flux increases); (3) within metabolite-mediated regulation, changes in [ADP] constitute the way through which most of the stimulation of oxidative phosphorylation is transmitted, while changes in [ATP] and [P_i] play only a minor role in this process.

As to the compensation of a decrease in oxygen concentration, the performed theoretical studies suggest that changes in the protonmotive force and reduction level of cytochrome *c* participate to a comparable extent in the compensation for the decrease in the flux through cytochrome oxidase. Without such a compensation, the decrease in the oxygen consumption flux at a given oxygen concentration would be several times greater than it is actually observed.

Acknowledgements

This work was supported by the KBN 4PO5D 05817 Grant.

References

[1] G.C. Brown, Biochem. J. 284 (1992) 1–13.
[2] W.M. Tylor, E. Van de Pot, L. Bygrave, Eur. J. Biochem. 155 (1986) 319–322.
[3] P.W. Hochachka, Muscles as Molecular and Metabolic Machines, CRC Press, Boca Raton, FL, 1994.
[4] G.A. Dudley, P.C. Tullson, R.L. Terjung, J. Biol. Chem. 262 (1987) 9109–9114.
[5] M.C. Hogan, P.G. Arthur, D.E. Bebout, P.W. Hochachka, P.D. Wagner, J. Appl. Physiol. 73 (1992) 728–736.
[6] P.G. Arthur, M.C. Hogan, P.D. Wagner, P.W. Hochachka, J. Appl. Physiol. 73 (1992) 737–743.
[7] G.P. Dobson, W.S. Parkhouse, J.M. Weber, E. Stuttard, J. Harman, D.H. Snow, P.W. Hochachka, Am. J. Physiol. 255 (1988) R513–R519.
[8] R.J. Rose, D.R. Hodgson, T.B. Kelso, L.J. McCutheon, T.A. Reid, W.M. Bayly, P.D. Gollnick, J. Appl. Physiol. 64 (1988) 781–788.
[9] G. Wegener, N.M. Bolas, A.A.G. Thomas, J. Comp. Physiol. B 161 (1991) 247–256.

[10] B. Chance, G.R. Williams, J. Biol. Chem. 217 (1955) 383–393.
[11] B. Chance, G.R. Williams, Adv. Enzymol. 17 (1956) 65–134.
[12] M.D. Brand, L.-F. Chien, P. Diolez, Biochem. J. 297 (1994) 27–29.
[13] A.K. Groen, R.J.A. Wanders, H.V. Westerhoff, R. van der Meer, J.M. Tager, J. Biol. Chem. 257 (1982) 2754–2757.
[14] B. Chance, S. Eleff, J.S. Leigh, D. Sokolow, A. Sapega, Proc. Natl. Acad. Sci. USA 78 (1981) 6714–6718.
[15] R.M. Denton, J.G. McCormack, FEBS Lett. 119 (1980) 1–8.
[16] R.G. Hansford, Curr. Top. Bioenerg. 10 (1980) 217–278.
[17] J.G. McCormack, A.P. Halestrap, R.M. Denton, Physiol. Rev. 70 (1990) 391–425.
[18] J.G. McCormack, R.M. Denton, Biochim. Biophys. Acta 1018 (1990) 278–291.
[19] V. Mildaziene, R. Baniene, Z. Nauciene, A. Marcinkeviciute, M. Morkuniene, V. Borutaite, B.N. Kholodenko, G.C. Brown, Biochem. J. 320 (1996) 329–334.
[20] B. Korzeniewski, W. Froncisz, Biochim. Biophys. Acta 1102 (1992) 67–75.
[21] B. Korzeniewski, Biochem. J. 330 (1998) 1189–1195.
[22] B. Korzeniewski, Biophys. Chem. 83 (2000) 19–34.
[23] B. Korzeniewski, W. Froncisz, Biochim. Biophys. Acta 1060 (1991) 210–223.
[24] B. Korzeniewski, Biophys. Chem. 57 (1996) 143–153.
[25] B. Korzeniewski, Biophys. Chem. 58 (1996) 215–224.
[26] B. Korzeniewski, Biophys. Chem. 59 (1996) 75–86.
[27] B. Korzeniewski, J.-P. Mazat, Biochem. J. 319 (1996) 143–148.
[28] E. Gneiger, R. Steinlechner-Maran, G. Méndez, T. Eberl, R. Margreiter, J. Bioenerg. Biomembr. 27 (1995) 583–596.
[29] D.F. Wilson, M. Ereciñska, C. Drown, I.A. Silver, Arch. Biochem. Biophys. 195 (1979) 485–493.
[30] D.F. Wilson, C.S. Owen, M. Ereciñska, Arch. Biochem. Biophys. 195 (1979) 494–504.
[31] D.F. Wilson, C.S. Owen, A. Holian, Arch. Biochem. Biophys. 182 (1977) 749–762.
[32] B. Korzeniewski, J.-P. Mazat, Acta Biotheor. 44 (1996) 263–269.
[33] F.R. Wiedemann, W.S. Kunz, FEBS Lett. 422 (1998) 33–35.
[34] H. Kacser, J.A. Burns, Symp. Soc. Exp. Biol. 32 (1973) 61–104.
[35] R. Heinrich, T.A. Rapoport, Eur. J. Biochem. 42 (1974) 89–95.
[36] C. Reder, J. Theor. Biol. 135 (1988) 175–201.
[37] B. Korzeniewski, W. Froncisz, Studia Biophys. 132 (1989) 173–187.
[38] T. Lettelier, M. Malgat, J.-P. Mazat, Biochim. Biophys. Acta 1141 (1993) 58–64.
[39] P. Mitchell, Nature 191 (1961) 144–148.
[40] H. Rottenberg, Biochim. Biophys. Acta 548 (1979) 225–253.
[41] H.V. Westerhoff, K. van Dam, Thermodynamics and control of free-energy transduction, Elsevier, Amsterdam, 1987.
[42] B.H. Groen, J.A. Berden, K. van Dam, Biochim. Biophys. Acta 1019 (1990) 121–127.
[43] E. Heinz, H.V. Westerhoff, K. van Dam, Eur. J. Biochem. 115 (1981) 107–113.
[44] R. Bohnensack, Biochim. Biophys. Acta 634 (1981) 203–218.
[45] R. Bohnensack, U. Küster, G. Letko, Biochim. Biophys. Acta 680 (1982) 271–280.
[46] F.N. Gellerich, R. Bohnensack, W. Kunz, Biochim. Biophys. Acta 722 (1983) 381–391.
[47] H.-G. Holzhütter, W. Henke, W. Dubiel, G. Gerber, Biochim. Biophys. Acta 810 (1985) 252–268.
[48] R.P. Hafner, G.C. Brown, M.D. Brand, Eur. J. Biochem. 188 (1990) 313–319.
[49] B. Korzeniewski, G. Brown, Biophys. Chem. 75 (1998) 73–80.
[50] M.K. Aliev, V.A. Saks, Biophys. J. 73 (1997) 428–445.
[51] R.S. Balaban, H.L. Kantor, L.A. Katz, R.W. Briggs, Science 232 (1986) 1121–1123.
[52] R.S. Balaban, Am. J. Physiol. 258 (1990) C377–C389.
[53] S. Kimura, T. Suzaki, S. Kobayashi, K. Abe, E. Ogata, Biochem. Biophys. Res. Commun. 119 (1984) 212–219.
[54] M.A. Titheridge, R.C. Haynes, Arch. Biochem. Biophys. 201 (1980) 44–55.
[55] T. Strzelecki, J.A. Thomas, C.D. Koch, K.P. LaNoue, J. Biol. Chem. 259 (1984) 4122–4129.
[56] J.M. Tager, A.K. Groen, R.J.A. Wanders, J. Duszyñski, H.W. Westerhoff, R.C. Vervoorn, in: R.A. Harris, N.W. Cornell (Eds.), Isolation, Characterisation and Use of Hepatocytes, Elsevier, Amsterdam, 1983, pp. 313–322.
[57] B. Korzeniewski, M.-E. Harper, M.D. Brand, Biochim. Biophys. Acta 1229 (1995) 315–322.
[58] E.K. Ainscow, M.D. Brand, Eur. J. Biochem. 265 (1999) 1043–1055.
[59] D. Duboc, M. Muffat-Joly, G. Renault, M. Degeorges, M. Toussaint, J.-J. Pocidalo, J. Appl. Physiol. 64 (1988) 2692–2695.
[60] K. Sahlin, Pflug. Arch. 403 (1985) 193–196.
[61] F.F. Jöbsis, W.N. Stainsby, Respir. Physiol. 4 (1968) 292–300.
[62] A.J. Godfraind, Physiology 223 (1972) 719–734.
[63] J.B.J. Chapman, Gen. Physiol. 59 (1972) 135–154.
[64] I.R. Wendt, J.B. Chapman, Am. J. Physiol. 230 (1976) 1644–1649.
[65] M. Tonkonogi, K. Sahlin, Acta Physiol. Scand. 161 (1997) 345–353.
[66] P. Andersen, B. Saltin, J. Physiol. 366 (1985) 233–249.
[67] M.L. Blei, K.E. Conley, M.J. Kushmerick, J. Physiol. 465 (1993) 203–222.
[68] F.W. Heineman, R.S. Balaban, Am. J. Physiol. 264 (1993) H433–H440.
[69] D.A. Harris, A.M. Das, Biochem. J. 280 (1991) 561–573.
[70] L.A. Katz, J.A. Swain, M.A. Portman, R.S. Balaban, Am. J. Physiol. 256 (1989) H265–H274.
[71] A.H.L. From, M.A. Petein, S.P. Michurski, S.D. Zimmer, K. Ugurbil, FEBS Lett. 206 (1986) 257–261.
[72] R.A. Kauppinen, J.K. Hiltunen, I.E. Hassinen, FEBS Lett. 112 (1980) 273–276.

[73] A.-M.L. Seymour, H. Eldar, G.K. Radda, Biochim. Biophys. Acta 1055 (1990) 107–116.
[74] F.M.H. Jeffrey, C.R. Malloy, Biochem. J. 287 (1992) 117–123.
[75] M.H.J. Eijgelshoven, J.H.G.M. van Beek, I. Mottet, M.G.J. Nederhoff, C.J.A. van Echteld, N. Westerchof, Circ. Res. 75 (1994) 751–759.
[76] M. Vendelin, O. Kongas, V. Saks, Am. J. Physiol. 278 (2000) C747–C764.
[77] M.D. Brand, M.P. Murphy, Biol. Rev. 62 (1987) 141–193.
[78] D.F. Wilson, in: A.N. Martinosi (Ed.), Membranes and Transport, Plenum Press, New York, 1982, pp. 349–355.
[79] T.M. Akerboom, H. Bookelman, J.M. Tager, FEBS Lett. 74 (1977) 50–54.
[80] J.J. Lemasters, A.E. Sowers, J. Biol. Chem. 254 (1979) 1248–1251.
[81] M. Ereciñska, M. Stubbs, Y. Miyata, C.M. Ditre, D.F. Wilson, Biochim. Biophys. Acta 462 (1977) 20–35.
[82] J.E. Hassinen, Biochim. Biophys. Acta 853 (1986) 135–151.
[83] M. Klingenberg, Angew. Chem. Int. Ed. 3 (1964) 54–61.
[84] D.E. Atkinson, Biochemistry 7 (1968) 4030–4034.