Virtual mitochondrial: towards an integrated model of oxidative phosphorylation complexes and beyond

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Abstract
The modelling of OXPHOS (oxidative phosphorylation) in order to integrate all kinetic and thermodynamic aspects of chemiosmotic theory has a long history. We briefly review this history and show how new ways of modelling are required to integrate a local model of the individual respiratory complexes into a global model of OXPHOS and, beyond that, into a reliable overall model of central metabolism.

Introduction
Mitochondria are cell organelles which play an essential role in the cell’s energy supply, providing much of the universal high-energy molecule, ATP, used in numerous energy-consuming processes throughout the cell. The core of ATP production, OXPHOS (oxidative phosphorylation), consists of four enzyme complexes (the respiratory chain) which, driven by redox reactions, establish a proton gradient over the inner mitochondrial membrane. This electrochemical gradient is then used by ATP synthetase to phosphorylate ADP into ATP.

History
OXPHOS has long been modelled in order to integrate all the kinetic and thermodynamic aspects of chemiosmotic theory [1]. The first significant models were developed in the framework of the NET-Model (non-equilibrium thermodynamic model) involving a linear dependence of the flux as a function of the thermodynamic forces [2,3]. In this framework, Stucki [4,5] described all the states of OXPHOS dynamics (State 4, State 3, uncoupled state, etc.) in terms of energy conversion with the use of phenomenological coefficients. He sought the optimal efficiency of the system and the maximal net rate of ATP synthesis, and calculated the degree of coupling under these conditions. Although OXPHOS arguably lies outside the linear domain around equilibrium, Stucki’s [4,5] description is simple and points to the fundamental parameters involved: degree of coupling, thermodynamic forces, rates, optimal efficiency, phenomenological stoichiometry, etc.

Similar models were derived by Pietrobon et al. [6] and Pietrobon and Caplan [7] to describe redox-driven proton pumps and ATP synthesis in mitochondria. These models were kinetic models, but, with the calculation of thermodynamic parameters, stressing the relationships between kinetic and thermodynamic parameters.

Bohnensack [8] was probably the first to derive a quantitative model involving nearly all the components of OXPHOS in an algebraic model. Using this model, groups in Magdeburg [9,10] and Amsterdam [11] discovered that the control of OXPHOS was shared by several steps as predicted by MCA (Metabolic Control Analysis) [12–15].

Holzhüttet al. [16] also used all the components of OXPHOS, but in a dynamic model based on ordinary differential equations applied to isolated rat liver mitochondria.

Korzeniewski and Froncisz [17] then developed a more comprehensive model also based on ordinary differential equations and thermodynamics, which was applied to isolated mitochondria or to intact tissues (muscle, heart and liver). The model was used to calculate the control coefficient in OXPHOS [18], to fit threshold curves in muscle [19], to predict the shape of threshold curves at low oxygen pressure [19] and to study the transition from rest to intensive work in muscle [20], leading to the concept of parallel activation. This model was also used by Korzeniewski et al. [21] to compare the threshold curves obtained with mitochondrial or nuclear DNA mutations.

All the above-mentioned models based on ordinary differential equations necessarily assume that the mitochondrial compartments (matrix, inner membrane and intermembrane space) are homogeneous. One of the first studies to take into account the organization and compartmentalization of OXPHOS inside the cell was the model of Aliev and Saks [22] on heart bioenergetics. The model was worked out by Vendelin et al. [23,24]. It takes into account the presence of creatine and adenylate kinase both in the inter-membrane space and in the cytosol to ensure the conversion of ATP synthesized in mitochondria into phosphocreatine, which diffuses in the cytosol and is then converted back into ATP.
where it is used. A backward diffusion of creatine terminates the functional cycle.

More recently, Cortassa et al. [25] developed an integrated model of cardiac mitochondrial energy metabolism and calcium dynamics. It includes a model of the tricarboxylic acid cycle, the transport of Ca\(^{2+}\) across the mitochondrial inner membrane and the regulation of dehydrogenases by Ca\(^{2+}\). The value of this model is that it uses the variations in intracellular and intramitochondrial Ca\(^{2+}\) to match the supply of mitochondrial energy to cellular demand.

With a systems biology approach and in the framework of the e-cell project, Yugi and Tomita [26] designed a model of 58 reactions linking 117 metabolites. They found 286 of 471 kinetic parameters in 45 articles. The model involves the respiratory chain, the tricarboxylic acid cycle, the \(\beta\)-oxidation of fatty acids, and the carrier of the inner mitochondrial membrane. Users can observe the time-course of enzyme activities and metabolite concentrations. The model is open to criticism for integrating too vast a set of parameters of different origin (human, bovine, pig, rabbit and rat) and tissue (heart, liver, etc.). A four-step scale of confidence is given which allows the user to apply the results obtained from the model with care when necessary.

Palsson's group also tackled the modelling of mitochondrial energetic metabolism from the structural and linear programming point of view: Ramakrishna et al. [27] studied the constraints imposed by the stoichiometry of biochemical reactions, especially the maintenance of an NADH steady state. FBA (flux-balance analysis) was used to characterize the optimal flux distributions for maximal ATP production. The model includes glycolysis, tricarboxylic acid cycle, lactate production or use and \(\beta\)-oxidation of fatty acids. Using FBA, they also characterized the metabolic behaviour due to genetic diseases, particularly the overproduction of metabolites from the tricarboxylic acid cycle as sometimes observed in clinical studies of mitochondrial pathologies. However, this study was performed under the assumption that ATP synthesis has to be maximal, which is not necessarily the case, and without taking into account the rate laws of the different steps of the mitochondrial network.

In a subsequent paper from the same group, Vo et al. [28] reconstructed the mitochondrial metabolism of the human cardiomyocytes based on proteomic and biochemical data. The metabolic network involves 189 reactions and 230 metabolites. FBA was applied to calculate the optimal flux according to three separate objective functions: ATP production, haem biosynthesis and phospholipid biosynthesis. In a further paper, the group used linear programming and uniform random sampling to identify steady states of the mitochondrial metabolism that “were consistent with the imposed physico-chemical constraints and available experimental data” [29]. They applied this analysis to the mitochondrial metabolism in diabetes, ischaemia and diet. Once again, the results of these studies clearly show the structural constraints existing in this metabolic network, but they do not take into account the reaction kinetics, which can profoundly modify the theoretically optimal production.

An integrated model of OXPHOS

Mitochondria are the target of many drugs that often act specifically on a given respiratory complex or on one of the enzymes of the ATP synthesis machinery. Examples are atovaquone, a hydroxynaphthoquinone antimalaria drug acting on complex III, MPTP (N-methyl-4-phenyl-tetrahydropyridine) acting on complex I inducing Parkinson’s disease, and cyanide on complex IV. Furthermore, respiratory complexes and/or enzymes of the ATP synthesis machinery [P\(_i\) transporter, ANT (adenine nucleotide transporter) and ATP synthase] can be affected by mutations affecting their properties and lead to mitochondrial diseases. It is important to understand how a local defect (enzyme deficiency) is expressed globally in mitochondrial oxygen consumption and/or ATP synthesis (see Figure 1).

To understand this link between local and global effects, a realistic model of OXPHOS is needed based on a ‘good’ model of the known kinetics of the isolated respiratory chain complexes. In other words, the kinetic of the respiratory complexes in the global model should represent their real behaviour when measured separately and not arbitrary functions, so that their variations can be monitored at the upper level of oxygen and/or ATP synthesis.

We have shown that several parameters have to be taken into account, especially the \(V_m\) and \(K_m\) for substrates and products. For this reason, the Law of Mass Action and net equations, which do not take into account these parameters, have to be replaced by more comprehensive equations such as a reversible Michaelis–Menten equation extended to several substrates and products. This led us: (i) to model the kinetics of the isolated respiratory chain complexes by an extended reversible Michaelis–Menten equation, valid for the in vivo range of substrate and product concentrations; (ii) to introduce the proton-motive force generated by the respiratory chain into these equations; and (iii) to incorporate these kinetic equations for individual complexes into a global model of OXPHOS.

Such a model would be a realistic model of the kinetics both of the isolated complexes involved in OXPHOS and of the whole OXPHOS itself. We would thus be able to compare the simulations obtained using this model with our experimental results from different tissues and in pathological cases of complex deficiencies. Such a model would be particularly useful for interpreting the threshold curves which express \(V_{O_2}\) or \(V_{ATP}\) as a function of the inhibition of a given respiratory complex [30–32]. They show a slow decrease in the flux, proportional to the control coefficient (on the ordinate) as a function of the inhibition of the step (on the abscissa) up to a threshold (usually \(>50\%\) inhibition), beyond which the inhibition is abrupt, reaching 0 at 100% inhibition. An important outcome of this study is that the thresholds vary, for the same respiratory complex, according to the tissue in the same organism. A model of
OXPHOS with a realistic representation of the kinetics of the individual complexes would be of great help in understanding the reasons for this tissue variation, i.e. how the threshold is related to the kinetic properties of the individual complexes (particularly in the case of complex deficiencies).

Stochastic modelling of the respiratory complexes

The respiratory complexes catalyse an intricate set of oxidation/reduction reactions coupling an electron pathway...
to extrusion of protons from the mitochondrial matrix. Because the crystallographic structures of most of the respiratory complexes are known, it is possible to model precisely the individual redox reactions.

A stochastic approach is particularly well adapted to describe the time course of the redox reactions that occur inside the respiratory chain complexes. Accordingly, we approach the molecular functioning of the $bc_1$ complex based on its known crystallographic structure and the midpoint potential of redox centres [33].

The main features of our simulations are the dominant and robust emergence of a Q-cycle mechanism [34] and the near absence of by-passes in the normal functioning of the $bc_1$ complex. However, this simple model fails to explain the inhibition of the $bc_1$ complex by antimycin and the accompanying increase in production of ROS (reactive oxygen species). To obtain inhibition, the return of the electron from the reduced haem $b_1$ to $Q_0$ must be blocked [35].

We also use this approach to describe the molecular functioning of the hydrophilic domain of complex I [36]. We show that most electrons take the route defined by NADH$^+$ site → FMN → N3 → N1b → N4 → N5 → N6a → N6b → N2 → Q site but frequently jump back and forth between neighbouring redox centres, with the result that the net flux of electrons through complex I is far smaller than the number of redox reactions that actually occur. We also hypothesize that the additional N1a redox centre could play a role in reducing the lifetime of the flavin semiquinone, thus limiting ROS production. However, the catalytic rate is higher and the level of reduction of the iron-sulphur centres is lower than what is observed. This indicates that a slower reaction or transconformation follows or is concomitant with the reduction of Q (H$^+$ pumping, change of conformation in the membranous and/or the hydrophilic arm of the complex I).

Conclusion: why model OXPHOS and metabolism in general?

Our modelling philosophy is certainly not to obtain a perfect simulation of experimental results at any price, a procedure usually involving an adjustment of the parameters unconcerned with reality. On the contrary, our aim is to collect all the experimental values and to check their self-consistency with the help of a model. For instance, in the case of OXPHOS, we measure oxygen consumption and ATP synthesis under different conditions and also assess the maximal activity of all the complexes of the respiratory chain on the same material. We also know their $K_m$ values for the different substrates and products. The question may thus be posed as follows: when all these kinetic properties are assembled in a dynamic system based on the individual rate equations, do we obtain the oxygen consumption or the ATP synthesis that we observe experimentally at a more global level?

It is obvious, however, that OXPHOS is not an isolated mechanism inside the cell. It is associated with several other pathways such as glycolysis, fatty acid metabolism, amino-acid synthesis and degradation through transporters and shuttles. Most of these elements are known and have been modelled. With the same approach in mind, they can be gathered together in a more global model whose self-consistency now becomes the main question.

In conclusion, in our opinion, the purpose of a model is not to fit experimental results accurately but rather to show inconsistencies that will lead to the unveiling of mechanisms or properties which were hitherto not taken into account or were even unknown.

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