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QUARTERLY

Is a mathematical model able to predict the result of a biochemical experiment?

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One of the criteria of a good scientific theory, proposed by Karl Popper, is prediction of the results of future experiments. This criterion, commonly realized in physics, is very difficult to be fulfilled in biochemistry because of great complexity of biological systems. The present article shows that, even in so sophisticated system as oxidative phosphorylation, an appropriate mathematical model is able to provide information what kind of an experimental result may be expected in a given, particular case, and thus to project an experiment being able to verify the model. Additionally, this article shows that intuitive interpretation of experimental results is frequently incorrect and therefore it is necessary to use quantitative mathematical methods.

The oxidative phosphorylation system can be divided into two subsystems: a subsystem producing the protonmotive force Δp (substrate dehydrogenation, respiratory chain) and a subsystem consuming Δp (ATP synthase, ATP/ADP carrier, phosphate carrier, ATP utilisation, proton leak). The simplest, intuitive, qualitative interpretation of experimental data concerning stimulation by hormones acting through an increase of cytoplasmic concentration of Ca^{2+} (vasopressin, glucagon, adrenaline) of the respiration rate in isolated hepatocytes had led to the conclusion that these hormones activate the Δp-producing subsystem exclusively [1]. The Δp-consuming subsystem would be in this formulation stimulated indirectly through an increase in [NADH], Δp and [ATP]. A similar opinion exists in the case of muscle contraction; there, calcium ions would stimulate directly the contraction and the related hydrolysis of ATP to ADP, and the latter compound would activate the reactions producing Δp.

On the other hand, a quantitative analysis performed using a dynamic mathematical model of oxidative phosphorylation [2, 3] suggested that both the reactions producing Δp and the reactions consuming Δp are activated to a very similar extent. This model showed that, for a realistic kinetics of the processes producing Δp and the processes consuming Δp, the activation by the mentioned hormones of the former processes exclusively would lead to a much greater increase in the reduction level of NAD and cytochrome *c*, in the [ATP]/[ADP] ratio as well as in Δp than it has been stated experimentally. The model also predicted that physiological concentrations of calcium ions activated the ATP/ADP carrier in liver.

The above mentioned discrepancy was the main reason for carrying out an experiment verifying the model and, particularly, for examining the influence of vasopressin on the oxidative phosphorylation system in isolated hepatocytes in the presence of lactate and pyruvate (unpublished). It was found that the membrane potential across the inner mitochondrial membrane was practically not changed after addition of this hormone and the resulting increase in the respiration rate. Thus, this experiment confirmed the predictions of the model and showed that the difference in activation of the two subsystems was only about 10%. Furthermore, just after publication of predictions of the model [3] our attention was

drawn to a paper which clearly suggested that the ATP/ADP carrier was stimulated by calcium ions [4]. This would be a further confirmation of the model predictions.

To elaborate quantitatively the experimental results obtained a new theoretical method, called "proportional activation approach" (unpublished) was used. This method enables calculation of a relative activation by an external effector (such as respiratory substrate, hormone or neural stimulation) of the subsystem producing Δp and the subsystem consuming Δp, on the basis of changes in the respiration rate and protonmotive force after addition of the effector. A proportional activation coefficient was defined as follows:

$$P_X^{AB} = \frac{\delta B/B}{\delta A/A} \quad (1)$$

where, in the case of oxidative phosphorylation, A is the Δp-producing subsystem, B is Δp-consuming subsystem, and $\delta A/A$ ($\delta B/B$) is a relative stimulation of the subsystem A (B) activity by an external effector X. When both subsystems are activated to a similar extent, the P coefficient is close to unity. When only subsystem A is activated, its value is equal to 0. The greater inequality of activation, the larger is the deviation of the P coefficient from unity.

The proportional activation approach can be applied both to design a new experiment and to elaborate already existing data, often very difficult to interpret intuitively. This method enabled to discover that not only vasopressin, but also other hormones acting through Ca^{2+}/cAMP (adrenaline, glucagon and others), respiratory substrates (lactate + pyruvate, fatty acids) as well as neural stimulation of muscle contraction (heart muscle and skeletal muscle) activate the Δp-producing and the Δp-consuming reactions to a similar extent (see Table 1). Particularly, the effectors acting through changes in cytoplasmic calcium ion concentration (hormones, neural stimulation of muscle contraction) exhibit a surprisingly good balance of stimulation (P coefficient close to 1). As the phenomenon of the well-balanced stimulation of both subsystems of oxidative phosphorylation seems to take place in a variety of different groups of external effectors, I have proposed (unpublished) an Equal Activation Rule (EAR) for this process and for bioenergetic systems generally. This rule states that external effectors increasing the flux through a given bioenergetic system and causing an increased chemical, mechanical, electric or osmotic work (but not heat production, e.g. uncoupling protein) activate different parts of this system to a similar extent. There are some evolutionary advantages of the EAR. The most important of them seems to be maintaining of internal homeostasis. Unequal activation of the Δp-producing and the Δp-consuming subsystem would lead to great changes in Δp as well as in the concentration of ATP and NADH. Many reactions essential for survival of a cell (e.g. protein synthesis, ion or substrate transport) utilise the above mentioned metabolites and therefore any greater change in their concentration would disturb the function of a cell as a whole. An equal activation of both subsystems seems to be, therefore, not only a fact well confirmed experimentally, but also a logical consequence of internal homeostasis.

Table 1

Proportional activation coefficients for different external effectors of oxidative phosphorylation (unpublished)

External effector	P coefficient
Lactate/pyruvate	0.64
Fatty acids	0.63
Vasopressin	1.10
Glucagon	about 1.0
Hormones acting through Ca^{2+}/cAMP (generally)	0.8 – 1.0
Neural stimulation (heart muscle)	1.06
Neural stimulation (skeletal muscle)	1.07

The results presented above are of great importance for the discussion on internal mechanisms of regulation of cell metabolism. In the past ten years, flux control coefficients and elasticity coefficients, defined in the frame of the Metabolic Control Theory [1, 5], were considered the most important parameters describing control and regulation of metabolic pathways. However, as both the Δp-producing and the Δp-consuming subsystems are activated by external effectors to a similar extent, the flux control coefficients for the two subsystems are of little significance. Regardless of their values, if both subsystems are activated n times, the flux through the entire system will increase n times as well. Thus, the internal control and regulation described by flux control coefficients and elasticity coefficients would be responsible only for compensation of little deviations from perfectly equal stimulation and for quenching of small internal fluctuations. For the same reasons, the state 4 → state 3 transition in isolated mitochondria, where hexokinase imposes an increased respiration rate to the entire system through a decrease in Δp and [ATP]/[ADP], is not a good model for the situation occurring in an intact cell. In skeletal muscle, for example, despite the fact that during contraction the respiration rate increases about 20 times, Δp, [ATP]/[ADP] and proton leak are relatively constant, so a cell still remains in the "physiological state" $3_{1/2}$ This forces a re-valuation of the present opinions about the control and regulation of bioenergetic pathways in the cell.

Summing up, the dynamic mathematical model of oxidative phosphorylation has predicted correctly that the hormones acting through calcium/cAMP stimulate both the Δp-producing and the Δp-consuming subsystem to a similar extent and that calcium ions activate the ATP/ADP carrier under physiological conditions. This model inspired the designing and performing an experiment which led to its verification. Finally, it led indirectly to formulation of the Equal Activation Rule for bioenergetic systems.

REFERENCES

1. Brand, M.D. & Murphy, M.P. (1987) *Biol. Rev.* **62**, 141–193.
2. Korzeniewski, B. & Froncisz, W. (1991) *Biochim. Biophys. Acta* **1060**, 210–223.
3. Korzeniewski, B. & Froncisz, W. (1992) *Biochim. Biophys. Acta* **1102**, 67–75.
4. Moreno-Sanchez, R., Houge, B.A. & Hansford, R.G. (1990) *Biochem. J.* **268**, 421–428.
5. Kacser, H. & Porteous, J.W. (1987) *Trends Biochem. Sci.* **12**, 5–14.