VINTAGE PAPER

Quantification of Flux Control in Metabolic Pathways

The current drive to develop sustainable processes for the production of fuels and chemicals involves development of efficient cell factories through metabolic engineering (Nielsen, 2001) where directed genetic modifications are performed with the objective of improving the properties of the cell factory. With the production of fuels and commodity chemicals, this typically involves redirection of the fluxes in the metabolic network such that high overall conversion yield to the desired product is obtained from the raw material, which is typically glucose.

In order to direct the genetic engineering, it is important to have tools for identification of metabolic engineering targets. Even though comprehensive metabolic models in recent years have shown good abilities to identify targets for metabolic engineering with the objective of redirecting the flux towards the product of interest, these models still do not allow for identification of flux-controlling enzymes in metabolic pathways.

The concept of flux-controlling enzymes in metabolic pathways was introduced by Kacser and Burns (1973) and Heinrich and Rapoport (1974), who independently derived a similar mathematical framework that allows for estimation of the so-called flux control coefficients in metabolic pathways. The flux control coefficients are essentially sensitivity coefficients that quantify the sensitivity of the flux through the pathway with respect to the concentration (or activity) of the individual enzymes. The same authors further derived other coefficients, such as elasticity coefficients for the enzymes, which are local properties of the enzymes that quantify the sensitivity of the individual enzyme catalyzed reactions with respect to the metabolite concentrations, and concentration control coefficients, which quantify how the concentration of the pathway metabolites changes in response to changes in the enzyme concentrations/enzyme activities.

The framework developed by Kacser and Burns and Heinrich and Rapoport was rapidly unified into the concept of Metabolic Control Theory, later changed into the more appropriate term Metabolic Control Analysis (MCA). The concept of MCA states that a regulated enzyme may not necessarily be the flux controlling enzyme in a pathway, a concept that at the time created some heated debates, but today it is generally accepted and MCA is used widely and it is now a concept covered by many textbooks on bioreaction engineering and metabolic engineering.

Since its introduction, the concept of MCA has been further developed and found use also in quantitative analyses of other biological processes. This development has mainly been driven by Prof. Hans Westerhoff and his research group and Prof. Douglas Kell and his research group which has also played an instrumental role, in particular in the initial development of the software GEPASI (now further developed by Prof. Pedro Mendes), that allows an easy use of the concept of MCA. The concept of MCA is quite simple for linear pathways, but the mathematical complexity increases when one considers realistic branched pathways.

The paper by Westerhoff and Kell (1987) published in *Biotechnology and Bioengineering* is an important contribution as it describes a concise representation, in the form of a single matrix equation, of all the equations linking the elasticity coefficients, the flux control coefficients and the concentration control coefficients. Using this concise matrix equation it is possible to calculate all the control coefficients in a metabolic pathway system from the elasticity coefficients. As the elasticity coefficients can be calculated from information about the enzyme kinetics, it is hereby possible to obtain information about how flux control is distributed if one has kinetic information about the individual enzymes.

Even though MCA is for obvious reasons very appealing—it is possible to estimate how flux is controlled in a pathway and hereby identify targets for metabolic engineering—it has found relatively little direct application in biotechnology, as it requires very extensive information about the kinetics. Despite its limited direct application, the MCA concept is however widely used, as the framework has clearly shown that there are differences between regulated enzyme and flux controlling enzymes. Through extensions of the concept, Prof. Westerhoff has given many good examples on how it is possible to dissect to which extent different processes contribute, in a quantitative fashion, to

Correspondence to: J. Nielsen, Department of Chemical and Biological Engineering, Chalmers University of Technology, Kemivagen 10, SE-412 96 Göteborg, Sweden; telephone: 46317728633; fax: 4631160062; e-mail: nielsenj@chalmers.se
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/bit.00000

overall function of complex biological systems (see, e.g., ter Kuile and Westerhoff, 2001). Furthermore, the philosophy of MCA plays an important role in understanding how metabolic pathways operate, and it has therefore become a central concept in metabolic engineering education.

Jens Nielsen
Professor of Systems Biology
Department of Chemical and Biological Engineering
Chalmers University of Technology
Goteborg, Sweden

References

Heinrich R, Rapoport TA. 1974. A linear steady state treatment of enzymatic chains. General properties, control and effector strength. Eur J Biochem 42:89–95.

Kacser H, Burns JA. 1973. The control of flux. Symp Soc Exp Biol 27: 65–104.

Nielsen J. 2001. Metabolic engineering. Appl Microbiol Biotechnol 55:263–283.

ter Kuile BH, Westerhoff HV. 2001. Transcriptome meets metabolome: Hierarchical and metabolic regulation of the glycolytic pathway. FEBS Lett 500:169–171.

Westerhoff HV, Kell DB. 1987. Matrix method for determining steps most rate-limiting to metabolic fluxes in biotechnology processes. Biotechnol Bioeng 30:101–107.