INTRODUCTION TO $^{13}$C METABOLIC FLUX ANALYSIS

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INTRODUCTION

Metabolic Flux Analysis (MFA)

In recent years, metabolic flux analysis has become one of the major tools in metabolic engineering (1-3). The aim of MFA is the detailed quantification of all metabolic fluxes in the central metabolic pathways of a microorganism. The result is a flux map that shows the distribution of anabolic and catabolic fluxes over the metabolic network. Figure 1 shows such a flux distribution for the simple example discussed throughout the text. More complex examples for quite different microorganisms can be found in (4-9). Based on such a flux analysis, the result of a genetic manipulation can be judged or possible targets for future genetic modifications might be identified. Thus MFA is also an important tool for genetic engineering.
Figure 1: Dynamics of a CLE illustrated by the example of a metabolic network from Figure 3. The isotopomer distribution over the network changes with time until it finally becomes stationary. The flux distribution assumed for this simulation run is indicated by the thickness of the arrows. The constant input labeling distribution of the experiment is shown at the top. At the beginning of the experiment only unlabeled molecules are present in the system. All pool sizes are set at 1 for illustrative purposes.

In principle, $^{13}$C MFA is a further development of the classic $^{14}$C tracer technique that was already used in the nineteen-thirties to elucidate the central biochemical pathways. The major difference is that $^{13}$C MFA is a quantitative method that does not only serve to prove the existence of a certain biochemical reaction step but also determines the flux in both directions of this step. This is valuable information for deciding whether it is worthwhile to knock out or overexpress a certain gene.

As a prominent example the MFA in (10) revealed a strong futile cycle in the anaplerosis of Corynebacterium glutamicum that is the same order of magnitude as the citric acid cycle flux. Thus a knock-out of one of the anaplerotic enzymes is a promising genetic manipulation in order to improve the metabolic capabilities of the organism. In fact the knock-out of PEP carboxykinase led to a significant increase in lysine production while the consequence of an overexpression was that the maximal growth rate was significantly lowered (11). This result has been verified by performing one MFA after each enzyme knock-out or overexpression and by comparing the results with those of the original strain. Clearly, such results cannot be produced by non-quantitative tracer experiments which can only prove the basic activity in the anaplerotic steps in each case.

Although MFA is an extremely useful tool for metabolic and genetic engineering it is a rather complicated procedure (12):