13C NMR Flux Ratio Analysis of Escherichia coli Central Carbon Metabolism in Microaerobic Bioprocesses

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Active pathways and catalytic capacities in microorganisms are often highly sensitive to the cell environment. For example, oxygen supply can have qualitative effects on biocatalytic configuration and rates.1,2 This paper addresses the behavior of wild-type E. coli MG1655 cells carrying a high copy number plasmid under microaerobic conditions (O2 < 0.02 mmol/L), a situation that is encountered frequently in nature as well as in the biotechnology industry.2,3 Only a few stable isotope labeling experiments have so far been pursued to investigate anaerobic and/or microaerobic metabolism.6–13 In this paper, information on active metabolic topology and carbon pathway flux ratios was obtained from the aforementioned E. coli strain under microaerobic conditions,14 which is a typical biocatalyst configuration, using biosynthetic fractional 13C-labeling of proteinogenic amino acids with a mixture of 10% [13C6]-glucose/90% unlabeled glucose as the sole carbon source and 2D [13C,1H]-correlation NMR spectroscopy for analysis of the resulting products.13,14 Analysis of the breakdown of the six-carbon skeleton of glucose from the 13C fine structures allows both determination of ratios of metabolic fluxes and an efficient analysis of the bioreaction network topology.13,15–17

Most 13C-labeling patterns which were observed for the microaerobic E. coli MG1655 system (Figure 1; Table 1; Table S1 in the Supporting Information) are similar to those previously reported for E. coli strain W3110 grown in a batch culture under strictly anaerobic conditions,13,18 indicating that the topology of active central metabolic pathways and the flux ratios are essentially the same under these microaerobic growth conditions14 as

![Figure 1](https://example.com/figure1.png)

**Figure 1.** 13C scalar coupling fine structures: (a) phenylalanine Cα, (b) alanine Cα, (c) aspartate Cα, (d) glutamate Cα, (e) histidine Cα. The fractionally 13C-labeled amino acids13,18 were obtained from wild-type E. coli cells grown as described in ref 14. Panels (a) to (e) derive from C2 of phosphoenolpyruvate (Pep), pyruvate (Pyr), oxaloacetate (Oa), 2-oxoglutarate (2-Og), and C3 of ribose-5-phosphate (R5P), respectively. In panel (e), the doublet of doublets arising from 13C2 of 2-oxoglutarate (2-Og) is further split by the two-bond scalar coupling J13C,H, which documents the presence of intact C5-fragments arising from the oxidative branch of the pentose phosphate pathway.13,16,17 The 2D [13C,1H]-COSY spectrum was recorded on a Bruker DRX 500 spectrometer.18 In each panel, the relative abundances (in %) of the different carbon fragments shown in panel (a) are indicated, where the preserved bonds from the source molecules are depicted in bold. These were calculated as described previously from the analysis of the 13C fine structure.15

under strictly anaerobic conditions (Figure 2). Thus, comparison of pyruvate and phosphoenolpyruvate 13C-labeling patterns13 reveals that 71% of the C1–C2 bonds in pyruvate have been reversibly cleaved at least once by pyruvate formate-lyase (Figure 1, a and b; Table 1).19 Consistent with this finding, formate is detected as the major byproduct in the culture medium.14 Since pyruvate formate-lyase is strongly suppressed by oxygen,19,20 its activity indicates that the intracellular concentration of free oxygen must be close to zero. The NMR data further show that oxaloacetate is synthesized solely via anaplerotic carboxylation of phosphoenolpyruvate (Figure 1c). The flux from 2-oxoglutarate to oxaloacetate is zero, showing that the tricarboxylic acid cycle is interrupted.13 This is typical for anaerobiosis, because strong repression