The phosphoenolpyruvate carboxykinase also catalyzes C₃ carboxylation at the interface of glycolysis and the TCA cycle of *Bacillus subtilis*

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Abstract

Quantitative physiological characterization and isotopic tracer experiments revealed that pyruvate kinase mutants of *Bacillus subtilis* produced significantly more CO₂ from glucose in the tricarboxylic acid cycle than is explained by the remaining conversion of phosphoenolpyruvate (PEP) to pyruvate catalyzed by the phosphotransferase system. We show here that this additional catabolic flux into the tricarboxylic acid cycle was catalyzed by the PEP carboxykinase. In contrast to its normal role in gluconeogenesis, PEP carboxykinase can operate in the reverse direction from PEP to oxaloacetate upon knockout of pyruvate kinase in a riboflavin-producing *B. subtilis* strain and in wild-type 168. At least in the industrial strain, we demonstrate the additional capacity of PEP carboxykinase to function as a substitute anaplerotic reaction when the normal pyruvate carboxylase is inactivated. Presumably as a consequence of the unfavorable kinetics of an ATP-synthesizing anaplerotic PEP carboxykinase reaction, such pyruvate carboxylase mutants grow slowly or, as in the case of wild-type 168, not at all.

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1. Introduction

The capacity of metabolic networks to compensate for mutations is referred to as genetic robustness (Dipple et al., 2001; Wagner, 2000) and is thought to be an important reason for barely detectable or silent phenotypes upon deletion of many metabolic genes (Fischer and Sauer, 2003a; Winzeler et al., 1999). Such network resilience to modifications hampers also rational engineering of central metabolism in applied contexts (Bailey, 1999) and two distinct mechanisms appear to be primarily responsible for robustness (Gu et al., 2003; Nowak et al., 1997; Wagner, 2000). First, redundancy is ensured by gene duplication, so that knockout of one gene is readily compensated for by one or more isoenzymes. Second, alternative pathways or genes with unrelated function become active and compensate for the loss-of-function.

Questions of robustness may be addressed experimentally by isotopic tracer experiments that allow metabolism-wide monitoring of network responses to perturbations (Christensen and Nielsen, 2000; Sauer, 2004; Szyperski, 1995; Wiechert, 2001; Wittmann, 2002). Such ¹³C-labeling methods for metabolic flux analysis revealed that knockouts of central metabolic enzymes were at least partially compensated when isoenzymes were present (Fischer and Sauer, 2003a). However, compensating alternative pathways were also demonstrated (Sauer et al., 2004; Zamboni and Sauer, 2003), in particular when the lesion involved components of the initial catabolic routes of glucose (Canonaco et al., 2001; Fischer and Sauer, 2003a, b; Flores et al., 2002; Jiao et al., 2003; Marx et al., 2003). A particularly well-studied case of flux rerouting involves pyruvate kinase mutants of *Escherichia coli*. While individual knockout of either of two isoenzymes is compensated, at least in part, by the other one (Fischer and Sauer, 2003a; Ponce...