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Mutation-induced metabolite pool alterations in *Corynebacterium glutamicum*: Towards the identification of nitrogen control signals

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Abstract

The influence of glutamate dehydrogenase activity on nitrogen regulation in *Corynebacterium glutamicum* was investigated. As shown by RNA hybridization experiments deletion of the *gdh* gene results in a rearrangement of nitrogen metabolism. Even when sufficiently supplied with nitrogen sources, a *gdh* deletion strain showed the typical nitrogen starvation response of *C. glutamicum*. These changes in transcription correlate with distinct alterations of intracellular metabolite pattern. Metabolite analyses of different mutant strains and the wild type indicated that ammonium and 2-oxoglutarate might influence the nitrogen regulation system of *C. glutamicum* cells.

Keywords: *Corynebacterium*; Glutamate dehydrogenase; Nitrogen control; Nitrogen regulation

1. Introduction

Corynebacterium glutamicum is a gram-positive soil bacterium belonging to the group of mycolic acid-containing actinomycetes (Chun et al., 1996). It was isolated by Kinoshita et al. (1957) in a screening program for L-glutamate-producing bacteria from a soil sample collected at Ueno Zoo in Tokyo and at that time designated as *Micrococcus glutamicus* (Udaka, 1960). Today, large amounts of L-glutamate (more than

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1,500,000 tonnes per year) and L-lysine (more than 560,000 tonnes per year) are produced by use of different *C. glutamicum* strains, in addition to smaller amounts of L-alanine, L-isoleucine and L-proline and in addition to different nucleotides (Leuchtenberger, 1996; Hermann, 2003).

Due to its pivotal role in glutamate production, basic biochemical work on the glutamate-producing enzymes of *C. glutamicum* was already started in the 1960s (Kimura, 1962; Oshima et al., 1964; Shiio and Ozaki, 1970; Tochikura et al., 1984). As most bacteria, *C. glutamicum* has two primary pathways to facilitate the incorporation of ammonium into the key nitrogen donors for biosynthetic reactions, L-glutamate and L-glutamine (Shiio and Ozaki, 1970; Jakoby et al., 1997; Tesch et al., 1998). Glutamate dehydrogenase (GDH) assimilates ammonium by the reductive amination of 2-oxoglutarate to L-glutamate. Alternatively, ammonium is assimilated *via* the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway, whereby ammonium is used to amidate L-glutamate under consumption of ATP to form L-glutamine by glutamine synthetase (GS). The amide group is then transferred reductively to 2-oxoglutarate by glutamate synthase (GOGAT), resulting in the net conversion of 2-oxoglutarate to L-glutamate. Assimilation *via* glutamate dehydrogenase is bioenergetically more favourable, as the GS/GOGAT pathway utilizes an additional mol ATP per mol ammonium assimilated. Consequently, GDH is preferentially used in ammonium-rich medium, while transcription of *gltBD*, coding for GOGAT, is completely repressed in this situation (Beckers et al., 2001; Schulz et al., 2002) and transcription of *glnA*, coding for GS, is maintained at a basal level to provide L-glutamine for growth (Nolden et al., 2001a). When the cells face nitrogen limitation, assimilation *via* GDH is not sufficient due to the low affinity of the enzyme. In this situation, expression of *glnA* and *gltBD* is activated and the GS/GOGAT pathway is preferentially used for ammonium assimilation.

In *C. glutamicum* the TetR-family transcriptional regulator AmtR was identified as the master regulator of nitrogen control (Jakoby et al., 2000; Beckers et al., 2005). Under conditions of high nitrogen supply AmtR blocks the transcription of nitrogen-controlled genes, e.g. *glnA* and *gltBD*. In response to nitrogen limitation, repression is relieved by an interaction of AmtR with the GlnK signal transduction protein (Beckers et al., 2005). Adenylylation of GlnK by the GlnD protein is crucial for derepression of transcription, since unmodified GlnK does not interact with AmtR (Beckers et al., 2005). In response to an improved nitrogen supply, GlnD deadenylylates GlnK again, resulting in repression of AmtR-controlled genes (Strösser et al., 2004). It is unknown how this regulation process is triggered in *C. glutamicum*, i.e. which metabolite(s) indicate(s) the nitrogen status of the cell and how this signal is transferred to the GlnD/GlnK/AmtR signal cascade.

In this study, we investigated the influence of glutamate dehydrogenase activity on nitrogen regulation in *C. glutamicum*. The *gdh* gene, encoding glutamate dehydrogenase, was isolated by Börmann et al. (1992) and mutant strains were characterized. Despite the high enzymatic activity of 1.3 U (mg protein)$^{-1}$ determined for *C. glutamicum* wild type GDH (Tesch et al., 1998), deletion of the *gdh* gene revealed astonishingly no detrimental effect on growth. Moreover, glutamate production was unaffected as well (Börmann et al., 1992; Börmann-El Kholy et al., 1993). Later, Tesch et al. (1998) observed a significantly enhanced activity of GS and GOGAT in the *gdh* deletion strain under nitrogen surplus, which might complement the lack of GDH activity. In this communication, we show that the activation of the GS/GOGAT pathway on expression level in a *gdh* deletion strain is triggered by characteristic changes in the metabolite pool of *C. glutamicum* leading to a nitrogen starvation response of the global nitrogen regulation network.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Strains and plasmids used in this study are listed in Table 1. Bacteria were routinely grown in Luria Bertani (LB) medium (Sambrook et al., 1989) at 30 °C (*C. glutamicum*) or 37 °C (*Escherichia coli*). LB medium for *C. glutamicum* strains was supplemented with 2% glucose (final concentration) and antibiotics were added in standard concentrations if appropriate (Ausubel et al., 1987). For cell culture, a standard inoculation scheme was applied. A fresh *C. glutamicum* culture in LB medium was used to inoculate minimal medium (Keilhauer et al., 1993) with an excess of nitrogen sources (approximately 500 mM ammonium)