MINI-REVIEW

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L-Glutamate and L-lysine: traditional products with impetuous developments

Received: 8 December 1998 / Received revision: 1 March 1999 / Accepted: 5 March 1999

Abstract Amino acids have been produced with the aid of microorganisms for nearly 40 years now. The economic importance of these cellular building blocks is enormous. Demand for them is rising continuously and currently more than 10^6 tonnes/year are required. Continual efforts to increase production performance are directed towards the microorganisms themselves, as well as towards technical improvements of the respective processes. A special position within the amino-acid-producing microorganisms is traditionally occupied by Corynebacterium glutamicum. Molecular research in conjunction with NMR studies of flux has revealed fascinating new properties of this particular organism, including the existence of a new type of exporter and reverse fluxes within the anaplerosis. The knowledge gained will enable the further improvement of production strains and furthermore extend fundamental insights into metabolite flux management within bacteria in general.

Introduction

More than four decades ago, in 1957, Dr. S. Udeka and Dr. S. Kinoshita in Japan discovered the bacterium Corynebacterium glutamicum and its particular property of excreting L-glutamate. Following this original discovery, mutant strains were developed to produce L-glutamate on individual scales of more than 500 m^3. C. glutamicum is a gram-positive bacterium that can be isolated from soil. Very closely related bacteria are Brevibacterium lactofermentum or Brevibacterium flavum (Liebl et al. 1991), Brevibacterium thiogenitalis and Corynebacterium melasceola. Together with genera like Streptomyces, Propionibacterium or Arthrobacter, C. glutamicum belongs to the actinomycetes subdivision of gram-positive bacteria. Its genome size is 3082 kb (Bathe et al. 1996), and its entire genome sequence is expected to be completed in 1999. The genetic tools for C. glutamicum, enabling gene-directed mutagenesis and transposon mutagenesis for example, are comparable to those available for Escherichia coli (Malumbres et al. 1995; Wohlleben et al. 1993; Vertes et al. 1994). Currently, effective strains for producing selected amino acids are available derived from C. glutamicum, E. coli, or Bacillus subtilis. Constant research on the flux properties of C. glutamicum reveal this organism to be an outstanding example of metabolic design. In this review we will present recent research results on the synthesis of L-glutamate and L-lysine with C. glutamicum. We will also include commercial aspects of the development of amino acid production.

The amino acid market

The amounts of amino acids currently produced can be seen in Fig. 1. The leader is still L-glutamate produced by C. glutamicum. This is followed by L-lysine and DL-methionine while the other amino acids trail behind. Figure 1 also shows the relationship between volume and price. Prices drop as a consequence of increased production volume, which leads to greater demand and competition and reduces prices even further. The increase in amino acid demand over the years is significant. Within just 10 years the total market has approximately doubled, with some amino acids displaying a particularly large increase (Fig. 2). For instance, the world market for L-lysine has increased more than 20-fold in the past two decades. Estimates assume that the market is currently increasing by 10%–15% per year. There is therefore considerable movement and...
The expansion of capital by the companies producing the amino acids. The reason for the increasing demand for L-lysine is that this essential amino acid is required as a feed additive for poultry and pig breeding. This increased use in animal feeds reflects a growing meat consumption, in particular in South America and China. Consequently, the requirement for further essential amino acids used as feed additives, like methionine, L-threonine and L-tryptophan, is also increasing. This is also true for the demand for vitamins. Part of the increased amino acid demand is due to increased pollution control since, with a balanced amino acid content in the feed, the manure contains less nitrogen.

The actual demand for the amino acids used as feed supplements can vary significantly. L-Lysine produced by fermentation competes with the natural L-lysine source soybean meal, where it is present in high concentrations. Since in 1997 the soybean meal market tightened globally, this led to an increased consumption of L-lysine made with *C. glutamicum*. This is also the case with methionine, where chemically made methionine competes with fish meal as the natural source of methionine. The worldwide decrease in sardine catches, caused by the El Niño phenomenon, resulted in a decreased availability of this natural methionine source. Thus amino acid consumption is influenced by livestock and feed production throughout the world and the prices of natural sources. In addition to the specific amino acids required as feed supplements, amino acids are used for a large variety of purposes, such as chemical building blocks, pharmaceutical products, or food supplements.

### A set of reactions at the anaplerotic node

As mentioned, both L-glutamate and L-lysine are made with *C. glutamicum*. Of course, a prerequisite for their intracellular synthesis is the constant supply of the respective citric acid cycle intermediates 2-oxoglutarate and oxaloacetate. Therefore, great attention has been paid to the anaplerotic reactions in *C. glutamicum*, and phosphoenolpyruvate carboxylase was considered to be of major importance to replenish the cycle. Surprisingly, use of the cloned phosphoenolpyruvate carboxylase gene (O'Regan et al. 1989; Eikmanns et al. 1989) and the study of an inactivation mutant revealed that this enzyme activity is not at all essential for growth or increased amino acid production (Peters-Wendisch et al. 1993). It was therefore initially assumed that the glyoxylate acid cycle could serve as an alternative anaplerotic route. However, a defined deletion mutant, devoid of isocitrate lyase, also showed unaltered amino acid production. Only $^{13}$C-NMR analyses, which enabled $^{13}$CO$_2$ incorporation to be traced, gave definite proof of the presence of a second carboxylating reaction in *C. glutamicum* (Peters-Wendisch et al. 1996). The investigation of this enzyme activity resulted in the detection of pyruvate carboxylase activity (Peters-Wendisch et al. 1997) and the cloning of its gene (Peters-Wendisch et al. 1998). This carboxylase activity had been hard to detect in the initial enzyme measurements (Tosaka et al. 1979; Jetten et al. 1995; Cocaing-Bousquet and Lindley 1995) since it is very unstable in crude extracts and requires an in situ enzyme assay using permeabilized cells instead of crude extracts for reliable determinations. Therefore, *C. glutamicum* has the pyruvate dehydrogenase shuffling acetyl-CoA into the citric acid cycle, and pyruvate carboxylase together with phosphoenolpyruvate carboxylase supplying oxaloacetate (Fig. 3). Indeed, the two carboxylases can substitute for each other during growth on glucose, but the pyruvate carboxylase is the more important reaction, since its deletion has an effect on growth. When both enzymes are deleted, growth on glucose is not possible, showing that these enzymes are the only relevant ones at the anaplerotic node. In addition to the activities of enzymes supplying the citric acid cycle with metabolites, oxaloacetate decarboxylase,