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Research review paper

ATP in current biotechnology: Regulation, applications and perspectives

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ABSTRACT

Adenosine tri-phosphate (ATP), the most important energy source for metabolic reactions and pathways, plays a vital role in the growth of industrial strain and the production of target metabolites. In this review, current advances in manipulating ATP in industrial strains, including altering NADH availability, and regulating NADH oxidation pathway, oxygen supply, proton gradient, the electron transfer chain activity and the F_0F_1-ATPase activity, are summarized and discussed. By applying these strategies, optimal product concentrations, yields and productivity in industrial biotechnology have been achieved. Furthermore, the mechanisms by which ATP extends the substrate utilization spectra and enhances the ability to challenge harsh environmental stress have been elucidated. Finally, three critical issues related to ATP manipulation have been addressed.

Contents

1. Introduction

An efficient and realistic strategy to achieve the three most important objectives in industrial biotechnology—the highest product concentration, the highest yield and the highest productivity of bulk or fine chemicals produced by microbial processes, is to manipulate the carbon and nitrogen flux in industrial strains. The rapid development of metabolic engineering provides the chance to achieve these objectives more rationally and efficiently (Bailey, 1991). However, more and more researchers are beginning to realize that overexpression, deletion, or introduction of heterologous genes in specific metabolic pathways does not always achieve these targets. A well-known instance is the glycolytic pathway. In both eukaryotic (Larsson et al., 1997) and prokaryotic microorganisms (Yokota et al., 1997), the flux through glycolytic pathway cannot be significantly increased by overexpression of genes encoding the key glycolytic

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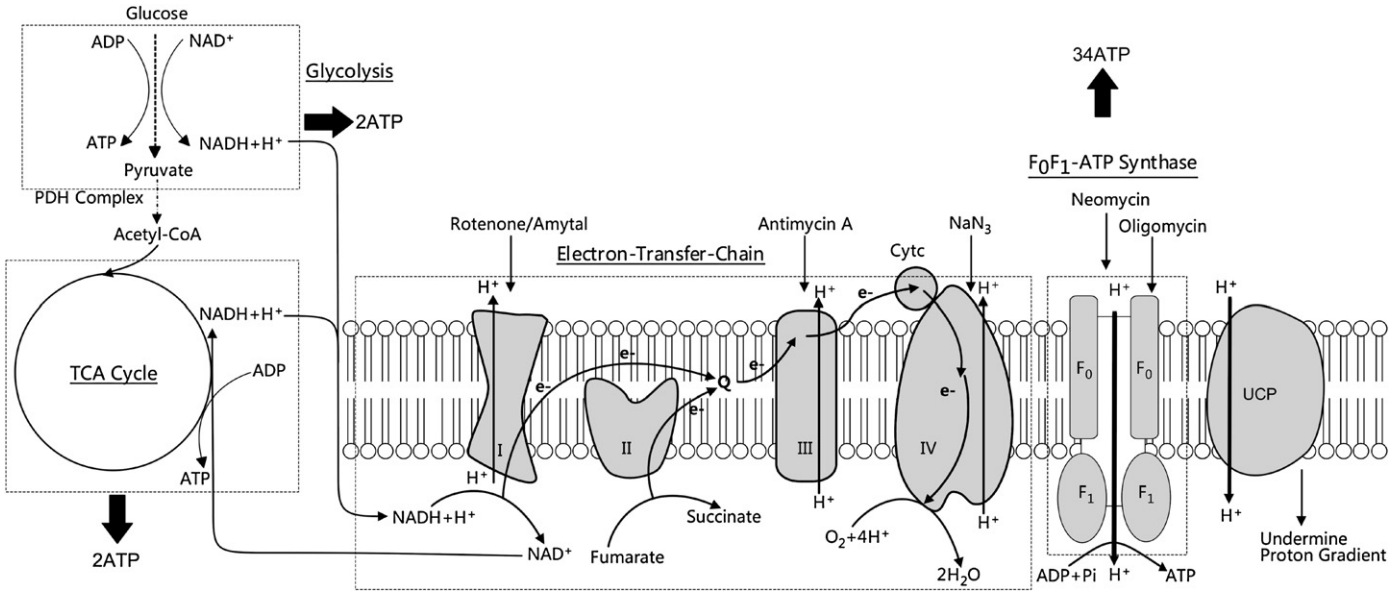

Fig. 1. ATP synthesis pathway and some representative inhibitors (Garcia-Martinez et al., 2001, Liu et al., 2006b; Nelson and Cox, 2004).

enzymes, either individually, or in combination. Subsequent studies demonstrated that the demand and the supply of ATP (adenosine triphosphate) played a key role in glycolysis (Larsson et al., 2000). It has been well-documented that cofactors such as ATP/ADP (adenosine di-phosphate) (Aoki et al., 2005; Liu et al., 2006a; Yokota et al., 1997), NADH (nicotinamide adenine dinucleotide-reduced)/NAD (nicotinamide adenine dinucleotide-oxidized) (Sanchez et al., 2005), and acetyl CoA (coenzyme A)/CoA (Lin et al., 2006), which are shared among metabolic pathways, played central roles in the distribution and rate of metabolic fluxes.

ATP, a kind of nucleotide, widely serves as substrate, product, activator or/and inhibitor in metabolic networks (Nelson and Cox, 2004). Based on these four basic functions, the demand and supply of ATP could affect active transportation (Yuroff et al., 2003), peptide folding (Kragol et al., 2001), subunit assembly (Kipnis et al., 2007), protein relocation and phosphorylation (Deutscher et al., 2006; Geissenhoner et al., 2001), cell morphology (Boldogh and Pon, 2006; Romero et al., 2007), signal transduction (Klipp et al., 2005; Mendum and Smith, 2002), and stress response (Hamilton et al., 2002; Klipp et al., 2005; Watanabe et al., 2005). Through these complicated physiology process, ATP is involved in many metabolic pathways and production of almost all of the metabolites by industrial strains. Therefore, the manipulation of ATP supply and demand could be a powerful tool to achieve specific biotechnological objectives, such as higher product concentration (Candela and Fouet, 2006), higher productivity (Causey et al., 2004; Jensen and Michelsen, 1992; Yokota et al., 1997) and higher yield on substrate (Harris et al., 2007), and even expand the substrate spectra (Wisselink et al., 2007) and enhance resistance to harsh environmental conditions (Sheng and Marquis, 2006; Shima et al., 2008; Watanabe et al., 2005).

Considering the importance of ATP in current biotechnology, and the observation that most previous reviews concerning ATP focused only on the structure and physiological function of F_0F_1-ATP synthase (Boyer, 1997; Nakamoto et al., 1999; Senior et al., 2002; Weber, 2007; Wittig and Schagger, 2008) and vacuole ATPase (Jefferies et al., 2008; Xiao et al., 2008), the present review is dedicated to strategies to manipulate ATP, their current and potential applications, and the future of ATP-oriented strategies in current biotechnology.

2. The regulatory strategies of ATP availability

The regeneration of ATP in microorganisms occurs in two ways, substrate-level phosphorylation and oxidative phosphorylation (Fig. 1). Manipulating oxidative phosphorylation seems to be a more efficient way to regulate the intracellular ATP concentration, because under aerobic conditions, most ATP production comes from oxidative phosphorylation pathway. It is conceivable that NADH availability, electron transfer chain (ETC), proton gradient, F_0F_1-ATPase and oxygen supply could all be regulatory candidates for manipulating the intracellular ATP availability.

2.1. Manipulating NADH availability and the oxidation pathway

Intracellular NADH, produced from glycolysis, fatty acid oxidation, and the citric acid cycle, can be converted to NAD in three separate ways. Under aerobic growth, NADH oxidation occurs through ETC, in which oxygen is used as the final electron acceptor, and a large amount of ATP is produced (Nelson and Cox, 2004). Under anaerobic growth and in the absence of an alternate oxidizing agent, the oxidation of NADH can occur by fermentative pathways, such as aldehyde dehydrogenase (Zhang et al., 2006), or lactate dehydrogenase (Zhu and Shimizu, 2004). In this case, energy production is mainly from substrate-level phosphorylation. NADH can also be directly oxidized into water and NAD through NADH oxidase (Vemuri et al., 2006; Vemuri et al., 2007). Therefore, manipulating the availability and oxidation pathway of NADH may be an efficient strategy to manipulate the intracellular ATP level.

There are three different strategies to manipulate the NADH availability to adjust the intracellular ATP content, based on NADH-related metabolic pathways. Firstly, manipulating NADH availability through overexpression or deletion of the key NADH related enzymes, such as *ackA* (acetate kinase) (Underwood et al., 2002), *aldA* (aldehyde dehydrogenase) (Zhang et al., 2006), *ldh* (lactate dehydrogenase) (Hols et al., 1999), and *pfl* (pyruvate formate-lyase) (Hasona et al., 2004). Secondly, supplementing the culture medium with specific substrates for NAD-dependent dehydrogenase, such as formate (Harris et al., 2007; Wang et al., 2007b), citrate (Sanchez et al., 2008; Wang et al., 2007a) and oxalate (Kim and Swartz, 2000). Finally, overexpression of NADH oxidase genes, such as *noxE* from *Lactococcus lactis* or *nox* from *Streptococcus pneumoniae*, that oxidize NADH into NAD and water without ATP regeneration (Vemuri et al., 2006; Vemuri et al., 2007).