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Research review paper

Vitamin B6 metabolism in microbes and approaches for fermentative production

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ABSTRACT

Vitamin B6 is a designation for the six vitamers pyridoxal, pyridoxine, pyridoxamine, pyridoxal 5′-phosphate (PLP), pyridoxine 5′-phosphate, and pyridoxamine. PLP, being the most important B6 vitamer, serves as a cofactor for many proteins and enzymes. In contrast to other organisms, animals and humans have to ingest vitamin B6 with their food. Several disorders are associated with vitamin B6 deficiency. Moreover, pharmaceuticals interfere with metabolism of the cofactor, which also results in vitamin B6 deficiency. Therefore, vitamin B6 is a valuable compound for the pharmaceutical and the food industry. Although vitamin B6 is currently chemically synthesized, there is considerable interest on the industrial side to shift from chemical processes to sustainable fermentation technologies. Here, we review recent findings regarding biosynthesis and homeostasis of vitamin B6 and describe the approaches that have been made in the past to develop microbial production processes. Moreover, we will describe novel routes for vitamin B6 biosynthesis and discuss their potential for engineering bacteria that overproduce the commercially valuable substance. We also highlight bottlenecks of the vitamin B6 biosynthetic pathways and propose strategies to circumvent these limitations.

Contents

1. Introduction

Vitamins are essential organic micronutrients in the diet of animals and humans. One of these, Vitamin B6, has been discovered almost one century ago (György, 1956; Hellmann and Mooney, 2010; Kraemer et al., 2012; Eggersdorfer et al., 2012). Vitamin B6 is a collective designation for the water-soluble vitamers: pyridoxal (PL), pyridoxine (PN), and pyridoxamine (PM), and their respective phosphate esters pyridoxal 5′-phosphate (PLP), pyridoxine 5′-phosphate (PNP), and pyridoxamine 5′-phosphate (PMP) (Fig. 1A; Rosenberg, 2012). PLP, the most important vitamer, serves as a cofactor for a wide variety of proteins and enzymes (Jansonius, 1998; Christen and Mehta, 2001; Eliot and Kirsch, 2004; Phillips, 2015). It has been estimated that >160 enzymes with distinct catalytic activities require vitamin B6 as a cofactor (about 4% of all described catalytic activities) (Percudani and Peracchi,

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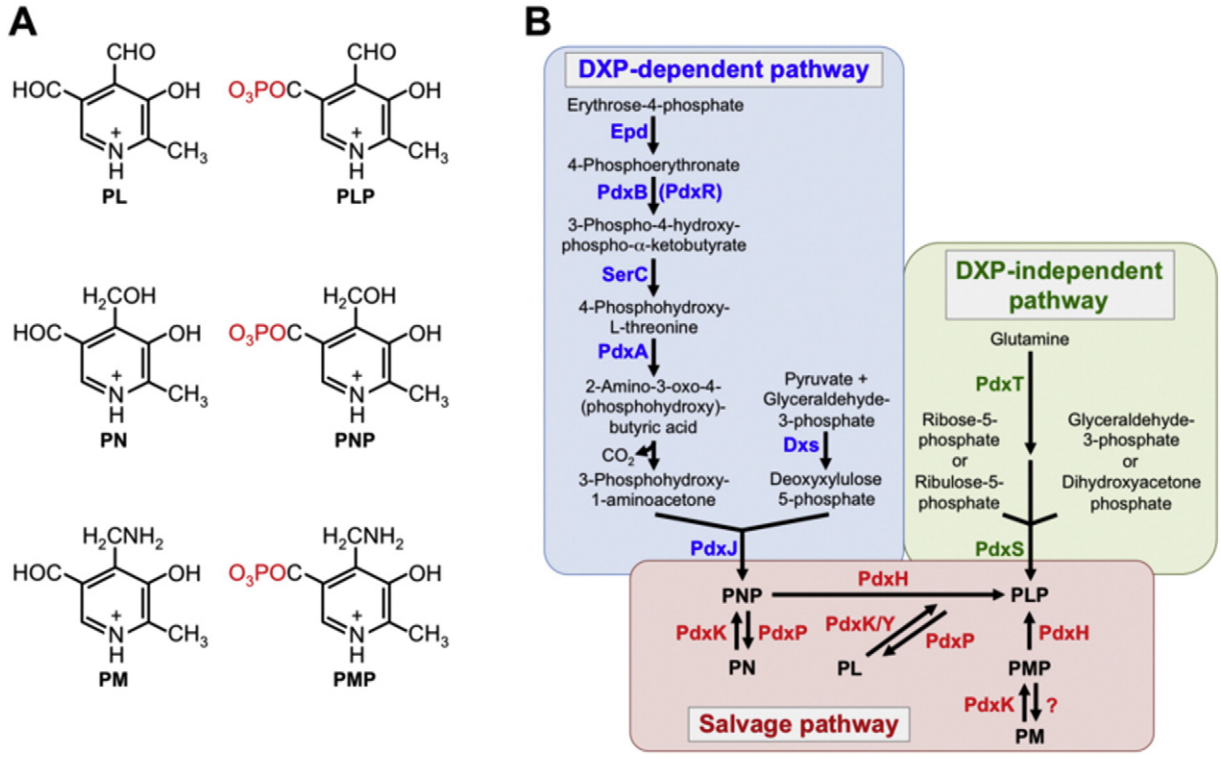

Fig. 1. (A) The B6 vitamers: pyridoxal (PL), pyridoxal 5′-phosphate (PLP), pyridoxine (PN), pyridoxine 5′-phosphate (PNP), pyridoxamine (PM), and pyridoxamine 5′-phosphate (PMP). (B) The deoxyxylulose 5-phosphate (DXP)-dependent and DXP-independent vitamin B6 biosynthetic routes and the salvage pathway for the interconversion of the B6 vitamers. Epd, erythrose 4-phosphate dehydrogenase; PdxB (PdxR), 4-phosphoerythronate dehydrogenase; SerC, 3-phosphoserine aminotransferase; PdxA, 4-phosphohydroxy-L-threonine dehydrogenase; PdxJ, PNP synthase; Dxs, 1-deoxyxylulose 5-phosphate synthase; PdxH, PNP oxidase; PdxS (PLP synthase subunit) and PdxT (glutaminase subunit) form the PLP synthase complex; PdxK, PL kinase present in *B. subtilis* and *E. coli*; PdxY, PL kinase present in *E. coli*. PdxK from *B. subtilis* has PN, PL, and PM kinase activity (Park et al., 2004).

2009). Most of the PLP-dependent enzymes are involved in biosynthesis of amino acids, decarboxylation and racemization reactions, cleavage of C_α–C_β bonds, α-elimination and replacement as well as β- and γ-elimination or replacement reactions (John, 1995; Eliot and Kirsch, 2004). Moreover, enzymes of deoxysugar biosynthetic pathways use PMP as a cofactor (Burns et al., 1996; Romo and Liu, 2011). It has also been shown that in both eukaryotes and prokaryotes PLP modulates the activity of DNA-binding transcription factors, among them regulators controlling vitamin B6 biosynthesis (Oka et al., 2001; Huq et al., 2007; El Qaidi et al., 2013; Belitsky, 2004a; Suvorova and Rodionov, 2016). Surveys of genome sequences revealed that about 1.5% of the genes of many free-living prokaryotes encode PLP-dependent proteins, underlining the importance of vitamin B6 for protein function and enzyme catalysis (Percudani and Peracchi, 2003). There is also evidence that vitamin B6 is implicated in oxidative stress responses (Bilski et al., 2000; Mooney and Hellmann, 2010; Moccand et al., 2014; Vanderschuren et al., 2013). Thus, vitamin B6 fulfils a variety of vital functions in different cellular processes (Mooney et al., 2009; Fitzpatrick et al., 2007; Vanderschuren et al., 2013).

For animals and humans, vitamin B6 has to be present in their diet because they are unable to synthesise this important micronutrient (Fitzpatrick et al., 2007, 2010; Kraemer et al., 2012). A reduced availability of vitamin B6 has been associated with several neurological disorders such as epileptic encephalopathy due to inherited errors in the enzymes interconverting B6 vitamers in the so-called "salvage pathway" (discussed in Section 2) (Mills et al., 2005; Bagci et al., 2008; di Salvo et al., 2012). Moreover, vitamin B6 deficiency can occur due to interactions between drugs, such as contraceptives, and enzymes of the salvage pathway or due to poor diet (Lumeng et al., 1974; Lussana et al., 2003; for a review di Salvo et al., 2011). Therefore, vitamin B6 is of commercial interest as a food additive and for applications in the pharmaceutical industry. In the food industry, the B6 vitamer PN in the hydrochloride form is usually used in combination with other vitamins in food products such as bakery products, cereals, baby nutrition, multivitamin juices and other beverages (Domke et al., 2005; Eggersdorfer et al., 2012). Vitamin B6 is also added to pet food and to the food that is used for intensive animal farming to improve animal health and to enhance the yield (Johnson et al., 1950; Verbeek, 1975, Eggersdorfer et al., 2012). In contrast to many studies and publications that report the positive effects of vitamin B6 and despite the large number of commercial products containing this compound, only very few reports of intoxication can be found. A recent case described photosensitive skin darkening, hyperemesis and diarrhoea as toxic effects, which disappeared soon after intoxication stopped (Cupa et al., 2015). Furthermore, long-time supplementation of pyridoxine in high doses is known to cause sensory neuropathy (Schaumburg et al., 1983, Albin et al., 1987). This effect is also used as a model for neuropathy (Hong et al., 2009, Potter et al., 2014).

Until now vitamin B6 is fully chemically synthesized through five principally different routes with variations, partly using expensive and/or toxic chemicals, like hydrogen cyanide, phosphorous pentoxide, and 1,4-butenediol (Pauling and Weimann, 1996; Kleemann et al., 2008, Eggersdorfer et al., 2012). Several attempts have been made by the biotech industry and academia to generate microorganisms for vitamin B6 production by classical mutagenesis and by genetic engineering (discussed in Section 2) (Pflug and Lingens, 1978; Ischikawa et al., 1997; Yocum et al., 2004; Hoshino et al., 2006a, 2006b, 2006c; Commichau et al., 2014, 2015). So far, none of the attempts were promising enough to establish an effective fermentation process. However, there is still considerable interest on the industrial side to shift from chemical synthesis processes to environmentally sustainable fermentation technologies.

This review intends to summarize recent findings regarding biosynthesis and homeostasis of vitamin B6. Moreover, we will describe the