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Review

Vitamin B_6 salvage enzymes: Mechanism, structure and regulation ☆

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ABSTRACT

Vitamin B_6 is a generic term referring to pyridoxine, pyridoxamine, pyridoxal and their related phosphorylated forms. Pyridoxal 5′-phosphate is the catalytically active form of vitamin B_6, and acts as cofactor in more than 140 different enzyme reactions. In animals, pyridoxal 5′-phosphate is recycled from food and from degraded B_6-enzymes in a "salvage pathway", which essentially involves two ubiquitous enzymes: an ATP-dependent pyridoxal kinase and an FMN-dependent pyridoxine 5′-phosphate oxidase. Once it is made, pyridoxal 5′-phosphate is targeted to the dozens of different apo-B_6 enzymes that are being synthesized in the cell. The mechanism and regulation of the salvage pathway and the mechanism of addition of pyridoxal 5′-phosphate to the apo-B_6-enzymes are poorly understood and represent a very challenging research field. Pyridoxal kinase and pyridoxine 5′-phosphate oxidase play kinetic roles in regulating the level of pyridoxal 5′-phosphate formation. Deficiency of pyridoxal 5′-phosphate due to inborn defects of these enzymes seems to be involved in several neurological pathologies. In addition, inhibition of pyridoxal kinase activity by several pharmaceutical and natural compounds is known to lead to pyridoxal 5′-phosphate deficiency. Understanding the exact role of vitamin B_6 in these pathologies requires a better knowledge on the metabolism and homeostasis of the vitamin. This article summarizes the current knowledge on structural, kinetic and regulation features of the two enzymes involved in the PLP salvage pathway. We also discuss the proposal that newly formed PLP may be transferred from either enzyme to apo-B_6-enzymes by direct channeling, an efficient, exclusive, and protected means of delivery of the highly reactive PLP. This new perspective may lead to novel and interesting findings, as well as serve as a model system for the study of macromolecular channeling. This article is part of a Special Issue entitled: Pyridoxal Phosphate Enzymology.

1. Introduction

Vitamin B_6 is a generic term which actually refers to the ensemble of six interconvertible pyridine compounds (vitamers): pyridoxine (PN, commonly known as vitamin B_6), pyridoxamine (PM), pyridoxal (PL) and their 5′-phosphorylated forms (PNP, PMP and PLP, respectively), which differ in the identity of the chemical group present at the 4′ position (Fig. 1). PLP is the biologically active and best known vitamer form, since it is used as enzyme cofactor in an enormous variety of biochemical transformations. In few enzymes, PMP also plays a catalytic role. In recent years, an additional function of B_6 vitamers as oxygen reactive species (ROS) scavengers and factors able to increase resistance to biotic and abiotic stress has been demonstrated in plants [1,2]. PLP and PN may also function as regulators of membrane ion transporters [3–5], and have been found to bind to steroid receptors [6] and to modulate transcription factors [7].

1.1. Biosynthesis and recycling of vitamin B_6

All living beings rely on vitamin B_6 for their existence, however, only microorganisms and plants are able to synthesize it *de novo*. All other organisms acquire vitamin B_6 from nutrients and interconvert its different forms in order to match their needs. Two independent *de novo* biosynthetic routes are known (Fig. 2) [8]. The first to be discovered was extensively studied in *Escherichia coli* and for a long time assumed to be ubiquitous. Nowadays, we know it is restricted to some eubacteria. This pathway, also called DXP-dependent pathway, is articulated in two branches which, starting from 4-phosphohydroxy-L-threonine (derived from erythrose 4-phosphate) at one end and from pyruvate and gliceraldehyde 3-phosphate at the other end, join in a ring closure reaction catalyzed by PNP synthase (coded by the

Abbreviations: PN, pyridoxine; PM, pyridoxamine; PL, pyridoxal; PNP, pyridoxine 5′-phosphate; PMP, pyridoxamine 5′-phosphate; PLP, pyridoxal 5′-phosphate; PLK, pyridoxal kinase coded by *PdxK* gene; PLK2, pyridoxal kinase coded by *PdxY* gene; PNPOx, pyridoxine (pyridoxamine) 5′-phosphate oxidase; NEE, neonatal epileptic encephalopathy; SHMT, serine hydroxymethyltransferase; AAT, aspartate aminotransferase

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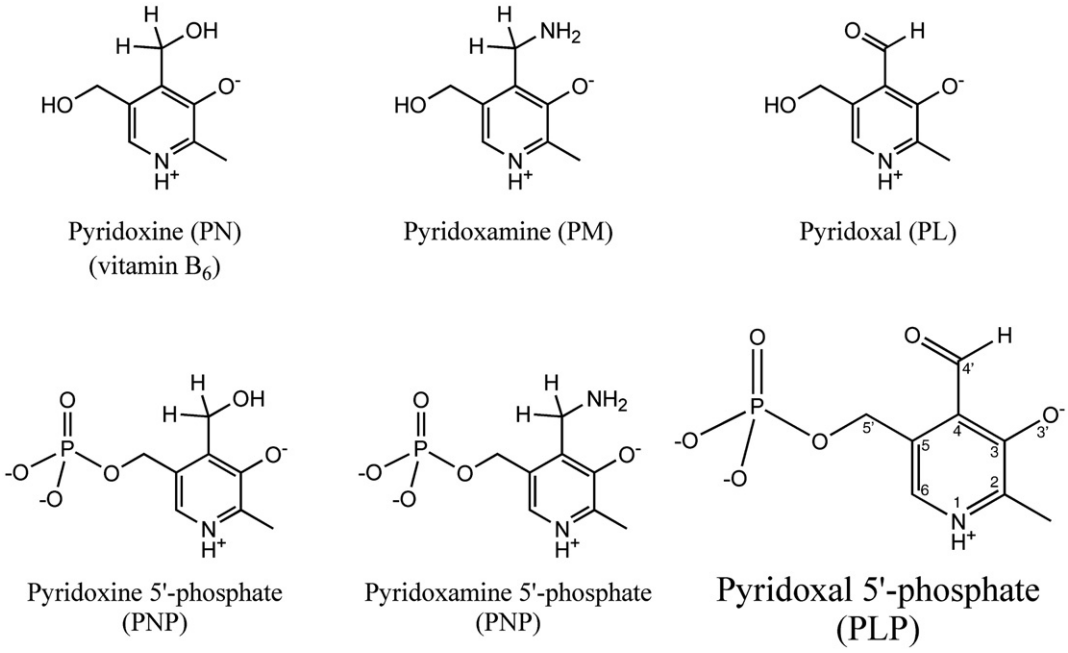

Fig. 1. Structures of the six B_6 vitamers. Carbon atom numbering is shown on the PLP structure.

PdxJ gene), which forms PNP, the first B_6 vitamer to be synthesized [9]. In the second route, the so-called DXP-independent pathway, PLP is directly formed from glutamine, either ribose or ribulose 5-phosphate and either glyceraldehydes 3-phosphate or dihydroxyacetone phosphate by the action of the PLP synthase complex (coded by the Pdx1 and Pdx2 genes) [10,11]. After the second route was serendipitously discovered in fungi, it became clear that it is much more widely distributed than the first one, being found in Archaea, most eubacteria and plants [2,12].

Humans, like all other mammals, obtain PLP from B_6 vitamers acquired from diet and recycled in a "salvage pathway" involving phosphatases, an ATP-dependent pyridoxal kinase (PLK) and a flavin mononucleotide (FMN)-dependent pyridoxine (pyridoxamine) 5′-phosphate) 5′-phosphate oxidase (PNPOx) (Fig. 2) [13]. PLK phosphorylates the 5′ alcohol groups of PN, PL and PM to form PNP, PLP and PMP respectively. PNP and PMP are further oxidized to PLP by PNPOx. PLP is largely present as such in meat, associated with glycogen phosphorylase in muscles, together with smaller amounts of PMP. PN, PNP and pyridoxine glucosides are the vitamers in plants. In mammals, ingested phosphorylated B_6 vitamers are first hydrolyzed to PL, PM and PN by intestinal phosphatase, while pyridoxine glucosides are hydrolyzed by a glucosidase prior to absorption. The absorbed vitamers are rapidly cleared, mainly by uptake into the liver, where they are phosphorylated by PLK, with PNP and PMP further oxidized to PLP by PNPOx. PLP re-enters the circulation bound to a lysine residue of albumin [14]. Delivery of active cofactor to the tissues, however, requires hydrolysis of circulating PLP to PL by the ecto-enzyme tissue nonspecific alkaline phosphatases [15]. A PLP specific phosphatase is also present, with essential role in cellular metabolism, especially in the brain where its level is substantially higher [16]. Once entered the cells, PL is re-phosphorylated by PLK and is somehow targeted to dozens of newly synthesized apo-B_6 enzymes.

1.2. Homeostasis of vitamin B_6 and human health

More than 140 different enzyme activities based on PLP are classified by the Enzyme Commission. They are distributed over five out of the six enzyme classes and represent 4% of all known catalytic activities [17]. PLP-dependent enzymes are not only involved in the synthesis, interconversion and degradation of amino acids but also play key roles in the metabolism of neurotransmitters, one-carbon units, biogenic amines, tetrapyrrolic compounds, amino sugars, modulation of steroid receptor-mediated gene expression and regulation of immune function. Of particular interest is the role of B_6 enzymes in brain metabolism, since the synthesis of several neurotransmitters, such as γ-aminobutyric acid (GABA), dopamine, epinephrine, norepinephrine, serotonin, serine, and histamine involves B_6 enzymes. Proper functioning of PLP-dependent enzymes and thus optimal health are dependent upon adequate levels of PLP in the cell.

PLP deficiency has been implicated in several neurological and non-neurological disorders. Dietary PLP insufficiency is quite rare since most dietary sources contain vitamin B_6. Major reasons for PLP deficiency can be attributed to malfunctioning of PLK and PNPOx, which may be caused either by inherited pathogenic mutations [15,18–23] or by drug induced inhibition [15,24–31]. The latter may produce symptoms such as unconsciousness, seizures, sleeplessness, headache, restlessness, agitation, tremors, and hallucination, while the former is implicated in severe pathologies, including neonatal epileptic encephalopathy, seizures, autism, Down syndrome, schizophrenia, autoimmune polyglandular disease, Parkinson's, Alzheimer's, epilepsy, attention deficit hyperactivity disorders and learning disability.

Very high levels of vitamin B_6 may have toxic effects [32–39]. PLP contains a very reactive aldehyde group at the 4′ position, that easily forms aldimines with primary and secondary amines and for this reason is often used as a protein labeling agent. The current recommended dietary allowance of vitamin B_6 is 2 mg/day in the United States. Toxicity is observed usually when the concentration exceeds 200 mg/day [40]. The levels of B_6 could also be raised as a result of an environmental insult or genetic defects. Toxicity of vitamin B_6 is known to cause sensory as well as motor neuropathies leading to numbness in hands and feet, that are usually reversible when supplementation is stopped [41].

The pool of free PLP *in vivo* is maintained at a very low level in the body, presumably to prevent toxic buildup. Regulation of PLP synthesis by PLK and PNPOx is a proposed homeostasis mechanism. Zhao and Winkler observed inhibition of *E. coli* PNPOx activities by product PLP, with a K_i of 8 μM [42]. In another study by our group, significant MgATP substrate inhibition of *E. coli* PLK was observed in the presence of PNP or PLP [43]. However, the most well established