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Review article

Intracellular trafficking of the pyridoxal cofactor. Implications for health and metabolic disease

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ABSTRACT

The importance of the vitamin B6-derived pyridoxal cofactor for human health has been established through more than 70 years of intensive biochemical research, revealing its fundamental roles in metabolism. B6 deficiency, resulting from nutritional limitation or impaired uptake from dietary sources, is associated with epilepsy, neuromuscular disease and neurodegeneration. Hereditary disorders of B6 processing are also known, and genetic defects in pathways involved in transport of B6 into the cell and its transformation to the pyridoxal-5'-phosphate enzyme cofactor can contribute to cardiovascular disease by interfering with homocysteine metabolism and the biosynthesis of vasomodulatory polyamines. Compared to the processes involved in cellular uptake and processing of the B6 vitamers, trafficking of the PLP cofactor across intracellular membranes is very poorly understood, even though the availability of PLP within subcellular compartments (particularly the mitochondrion) may have important health implications. The aim of this review is to concisely summarize the state of current knowledge of intracellular trafficking of PLP and to identify key directions for future research.

Contents

1. Introduction

The discovery of the B6 cofactor (pyridoxal 5'-phosphate, PLP) more than seventy years ago [1–3] initiated decades of fundamental biochemical research that has defined the unique role of that cofactor in enzyme catalysis [4–6], including activation of amine functional groups in metabolites [7,8] and, in the case of

Abbreviations: MCP, mitochondrial carrier protein; PLP, pyridoxal 5′-phosphate; PN, pyridoxine; PL, pyridoxal; PM, pyridoxamine; PMP, pyridoxamine 5′-phosphate; ALAS, 5-aminolevulinate synthase; ITC, isothermal titration calorimetry.

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glycogen phosphorylase, activation of inorganic phosphate for phosphorolytic cleavage of glycosidic bonds [9]. PLP-dependent enzymes are now known to be an extraordinarily diverse family performing many essential metabolic functions in amino acid biosynthesis and catabolism, 1-carbon metabolism, membrane lipid biosynthesis, production of neurotansmitters and biogenic polyamines, as well as glycogen cycling and iron metabolism (iron-sulfur cluster and heme biosynthesis) (Table 1). While only PLP (and its congener, pyridoxamine 5'-phosphate (PMP)) can directly function in catalysis, the extended B6 family includes multiple vitamer forms, differing in phosphorylation state and modification of the 4′ carbon (Fig. 1).

The biosynthesis and metabolic interconversion of these vitamers has recently been worked out in detail, revealing two major biosynthetic pathways [10–13]. Vertebrates (including humans) lack both pathways, and thus rely on a salvage pathway to convert B6 provided by dietary sources or commensal intestinal flora into the enzyme cofactor, PLP. While not all organisms are able to synthesize the B6 cofactor, the ability to utilize environmental B6 through efficient uptake and salvage pathways appears to be universal [14]. Localization of these pathways has important implications for cellular metabolism. In particular, the exclusive localization of the salvage pathway to the cytoplasm of eukaryotic cells makes delivery of the membrane-impermeable PLP cofactor to enzymes in other subcellular compartments (particularly the mitochondrion) critically dependent on efficient mechanisms for *intra*-cellular cofactor trafficking. This requirement for intracellular PLP transport systems has been generally neglected and represents an important area for future research.

2. Uptake at the plasma membrane

The B6 story starts at the cell surface. Utilization of environmental sources of B6 is a universal feature of life, and specific transport systems exist for cellular uptake of B6 vitamers (Fig. 2). In the yeast *Saccharomyces cerevisiae*, Tpn1p, a member of the purine-cytosine permease subfamily in the major facilitator superfamily, has been identified as the plasma membrane pyridoxine (vitamin B6) transporter [15,16]. Transport studies have demonstrated that Tpn1p is a unique, high affinity ($K_m = 0.55$ μM) pyridoxine carrier with broad substrate specificity for unphosphorylated B6 vitamers (including pyridoxine (PN), pyridoxamine (PM), and pyridoxal (PL)), utilizing a proton symport mechanism to concentrate B6 in the cell. Tpn1p is an integral membrane protein, predicted to be comprised of 12 transmembrane regions based on hydropathy analysis of the sequence.

Two homologous transport systems have been found in human cells [17–19], with distinct substrate specificity patterns. One

Table 1
Subcellular localization of pyridoxal 5'-phosphate dependent enzymes in *Saccharomyces cerevisiae*.

Enzyme Name	Abbreviation	EC no.	Gene ID[a]	Genetic locus[a]
1. Cytoplasm				
Aspartate aminotransferase	AAS	2.6.1.1	Aat2	YLR027C
Aromatic aminotransferase I	ArAT	2.6.1.57	Aro8	YGL202W
Aromatic aminotransferase II	ArAT	2.6.1.57	Aro9	YHR137W
7,8-Diamino-pelargonic acid aminotransferase	DAPA	2.6.1.62	Bio3	YNR058W
Kynurenine aminotransferase	KAT	2.6.1.7	Bna3	YJL060W
Kynureninase	KYNU	3.7.1.3	Bna5	YLR231C
L-ornithine transaminase	OAT	2.6.1.13	Car2	YLR438W
Cystathionine γ-lyase	CTH	4.4.1.1	Cys3	YAL012W
Cystathionine β-synthase	CBS	4.2.1.22	Cys4	YGR155W
Dihydrosphingosine phosphate lyase		4.1.2.27	Dpl1	YDR294C
Glutamate decarboxylase	GAD	4.1.1.15	Gad1	YMR250W
Glycogen phosphorylase	GP	2.4.1.1	Gph1	YPR160W
Histidinol-phosphate aminotransferase		2.6.1.9	His5	YIL116W
Cysteine-S-conjugate β-lyase		4.4.1.13	Irc7	YFR055W
Serine palmitoyltransferase		2.3.1.50	Lcb1	YMR296C
Serine palmitoyltransferase		2.3.1.50	Lcb2	YDR062W
Bifunctional cysteine synthase/O-acetylhomoserine aminocarboxypropyltransferase		2.5.1.47	Met17	YLR303W
3-Phosphoserine aminotransferase	PSAT	2.6.1.52	Ser1	YOR184W
Serine hydroxymethyltransferase	SHT	2.1.2.1	Shm2	YLR058C
Branched-chain amino acid aminotransferase	BCAT	2.6.1.42	Bat2	YJR148W
γ-aminobutyrate (GABA) transaminase		2.6.1.19	Uga1	YGR019W
2-Aminoadipate transaminase	AadAT	2.6.1.39		YER152C
Ornithine decarboxylase	ODC	4.1.1.17	Spe1	YKL184W
S-adenosylmethionine decarboxylase	AdoMetDC	4.1.1.50	Spe2	YOL052C
Phosphatidylserine decarboxylase	PSD	4.1.1.65	Psd1	YNL169C
2. Mitochondria				
Aspartate aminotransferase	AAT	2.6.1.1	Aat1	YKL106W
Alanine:glyoxylate aminotransferase	AGAT	2.6.1.44	Agx1	YFL030W
Alanine transaminase	ALT	2.6.1.2	Alt1	YLR089C
Acetylornithine aminotransferase		2.6.1.11	Arg8	YOL140W
Kynurenine aminotransferase	KAT	2.6.1.7	Bna3	YJL060W
L-serine (L-threonine) deaminase (catabolic)		4.3.1.19	Cha1	YCL064C
Glycine decarboxylase complex	GCC	2.1.2.10	Gcv2	YMR189W
5-Aminolevulinate synthase	ALAS	2.3.1.37	Hem1	YDR232W
Threonine deaminase	TD	4.3.1.19	Ilv1	YER086W
Cysteine desulfurase		2.8.1.7	Nfs1	YCL017C
Serine hydroxymethyltransferase	SHT	2.1.2.1	Shm1	YBR263W
Branched-chain amino acid aminotransferase	BCAT	2.6.1.42	Bat1	YHR208W
3. Peroxisome				
Alanine:glyoxylate aminotransferase	AGAT	2.6.1.44	Agx1	YFL030W

[a] Gene ID and genetic locus from *Saccharomyces cerevisiae* genome data.