Update on Interconversions of Vitamin B-6 with Its Coenzyme[1,2]

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ABSTRACT Biosynthesis of pyridoxal 5′-phosphate (PLP) depends upon the relatively specific action of two consecutive enzymes, viz. pyridoxal (pyridoxine, pyridoxamine) kinase and pyridoxine (pyridoxamine) phosphate oxidase. Less specific phosphatases catalyze hydrolyses of the 5′-phosphates of the vitamers pyridoxal, pyridoxamine, and pyridoxine. From the recognition a generation ago of these processes by which the three forms of vitamin B-6 and their 5′-phosphates are interconverted, more recent studies have provided a fairly sophisticated understanding of the molecular characteristics of the enzymes involved. The evolutionary retention of homologous portions of pyridoxal kinase in humans as well as bacteria and the most recent finding of a highly conserved region of the pyridoxine (pyridoxamine) phosphate oxidase, also from both prokaryotic and eukaryotic organisms, emphasize the importance of these catalysts in the formation of a coenzyme that is essential for most organisms. Both kinase and oxidase involved in B-6 metabolism are potential targets for pharmacologic agents. J. Nutr. 129: 325–327, 1999.

KEY WORDS: • Vitamin B-6 • pyridoxal kinase
• pyridoxine (pyridoxamine) 5′-phosphate oxidase
• pyridoxal 5′-phosphate

By the 1960s it was clear that the three, natural, free forms of vitamin B-6, namely pyridoxine (PN),[4] pyridoxal (PL), and pyridoxamine (PM), could be routed to the principal operating coenzyme pyridoxal 5′-phosphate (PLP) by the actions of two types of enzymes, a kinase that catalyzes phosphorylation of the 5-hydroxymethyl group of all three of the vitamers and an oxidase that catalyzes oxidation of the 5′-phosphate of pyridoxine (PNP) and of pyridoxamine (PMP). Additionally recognized were the phosphatases that catalyze hydrolytic reversions of the vitaminic phosphates to restore the free vitamers. The interactions involved are shown in **Figure 1**. With minor modifications to indicate other metabolic events (Ink and Henderson 1974, McCormick 1989, Snell and Haskell 1971), this scheme was used to illustrate the central role of both the kinase and oxidase in B-6 metabolism. Recent encyclopedic coverage on both vitamin and coenzyme forms of B-6 was published elsewhere (McCormick 1996 and 1997).

The first significant purifications and characterizations of the pyridoxal kinases responsible for catalyzing the phosphorylation of PN and PM, as well as PL from both prokaryotic and eukaryotic sources, revealed that most higher organisms preferred Zn^{2+} rather than Mg^{2+} as the ATP chelated cosubstrate and indicated additional activation by K^+ (McCormick and Snell 1959, McCormick et al. 1961). Confirmation of the stimulatory effect of K^+ was recently reported for the kinase in human erythrocytes (Laine-Cessac and Allain 1996). Pharmacologically interesting expansions of earlier studies that demonstrated the inhibition of pyridoxal kinase by carbonyl reagents (McCormick and Snell 1959 and 1961, McCormick et al. 1960) are the recent findings that the mammalian kinase is also a benzodiazepine-binding protein (Hanna et al. 1997). Drugs that inhibit pyridoxal kinase have now been divided into three groups, viz. substrate competitive types, such as theophylline and progabide; those that form covalent complexes, such as cycloserine, dopamine, isoniazid, and thiamphenicol glycinate; and a third somewhat related group comprised of levodopa, D-penicillamine, and muzolimine (Laine-Cessac et al. 1997).

Most recently, it was shown that *Escherishia coli* has two kinases for B-6, viz. one (classic) using all three vitamers and another that uses only pyridoxal as substrate (Yang et al. 1998). It appears both function in the salvage pathway of PLP biosynthesis. The direct formation of PLP in *E. coli*, unlike in mammals, can be achieved via 4-phospho-hydroxy-L-threonine with ring closure to pyridoxine 5′-phosphate (Zhao and Winkler 1996). In such a case, PNP would be oxidized to PLP by the oxidase, and the kinases would be used to recover PL and PN after hydrolytic cleavages.

An oxidase activity that catalyzes the second step in the formation of PLP from PNP and PMP was noted in earlier work with rabbit livers (Pogell 1958, Wada and Snell 1961). The first complete purification of pyridoxine (pyridoxamine) 5′-phosphate oxidase from liver, and its certain characterization as an FMN-dependent enzyme (Kazarinoff and McCormick 1975), was followed by a fairly extensive delineation of its properties (McCormick and Merrill 1980), including the amino acid residues that may be involved in binding of the coenzyme and are likely participating in the catalytic mechanism (Bowers-Komro and McCormick 1984). The oxidase was isolated from other tissues and other organisms, but its general characteristics are similar even at the level of *E. coli* (Zhao and Winkler 1995). With the sequences of pyridoxine (pyridoxamine) 5′-phosphate oxidase from several sources available, and using computer techniques applied in molecular biology, by comparing the primary sequences from various organisms, it was feasible to find out more information about this enzyme. This was done in a few cases with microorganisms (Loubbardi et al. 1995); however, extending this comparison to more eukaryotic species would ultimately allow a better understanding of the enzyme.

Given the considerable base of information we had derived for the higher eukaryotic oxidase, and the fact we recently obtained a cDNA library from *Schizophyllum commune* during our work to obtain the cDNA sequence for a riboflavin side-chain oxidizing enzyme (Chen and McCormick 1997), it

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[4] Abbreviations used: FMN, flavin mononucleotide; ORF, open reading frame; PL, pyridoxal; PLP, pyridoxal 5′-phosphate; PM, pyridoxamine; PMP, pyridoxamine 5′-phosphate; PN, Pyridoxine; PNP, 5′-phosphate of pyridoxine.

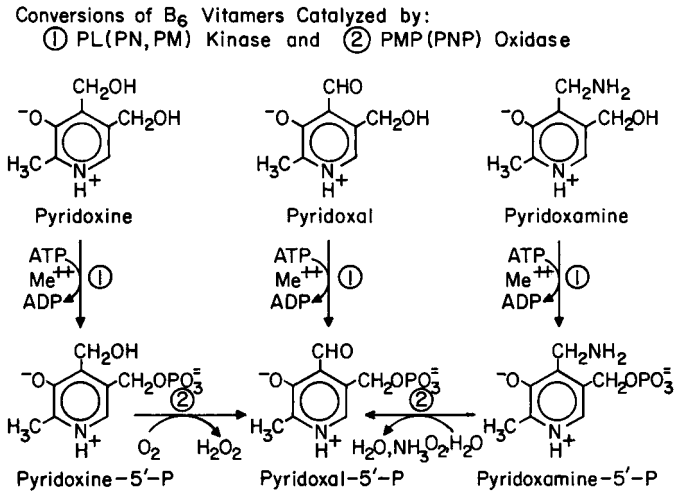

FIGURE 1 Interconversions of B-6 vitamers with their 5′-phospho forms including the coenzyme PLP.

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  1  GGTCGAATCG TATGGGTTCA TAGTCGATCC TGTAATTCGT AGAGTGACTA
 51  GTGTGAAATT GCGATACGTA GGGTCGGAAC CGGCCTCCAG ATCAAGCAAC
101  ATGGCGACCG AGTGCACCCA TCAGTGCACC AAGACGGGCG AGATCGCCCA
       M  A  T  E     C  T  H     Q  C  T     K  T  G  E     I  A  Q      17
151  GAAGATGCTC CTCAGAGACA ACTACCAGGC AGGTGGCCTC AACGAAGCGC
       K  M  L     L  R  D  N     Y  Q  A     G  G  L     N  E  A  L      34
201  TGACCGACGA TCCGATCGAT CAGTTCACCA AGTGGTTCAA GGAGGCCATC
       T  D  D     P  I  D     Q  F  T  K     W  F  K     E  A  I         50
251  GAGGACGCCC GTGAGACCCT GCCGAATGCT ATCACGCTCT CCTCGGCCGA
       E  D  A  R     E  T  L     P  N  A     I  T  L  S     S  A  D      67
301  CGAGTCGGGC CGTCCTTCGT CGCGTATGGT CCTCCTGAAG GAGCTCGATG
       E  S  G     R  P  S  S     R  M  V     L  L  K     E  L  D  E      84
351  AGCGTGGCTT CGTCTTCTAC TCCAACTATG GGAGCCGTAA GGGCCATTGC
       R  G  F     V  F  Y     S  N  Y  G     S  R  K     G  H  C         100
401  ATCGCGGCGA ATCCGAACGC GGCACTTGTT TTCTTCTGGA AGGCGCTCGA
       I  A  A  N     P  N  A     A  L  V     F  F  W  K     A  L  E      117
451  GCGTCAGGTG AGAGTCGAGG GCATCGTCGA GCATGTCCCG CGTGAGACCT
       R  Q  V     R  V  E  G     I  V  E     H  V  P     R  E  T  S      134
501  CCGACGCTTA CTTCAAGACC AGAGCGCGTG GCTCCAAGCT CGGCGCATGG
       D  A  Y     F  K  T     R  A  R  G     S  K  L     G  A  W         150
551  GCGTCCCGTC AGTCCGATGT TATCAAGAAC AGACAGGAGC TCGACGAGCT
       A  S  R  Q     S  D  V     I  K  N     R  Q  E  L     D  E  L      167
601  CACCGCTAAG AATACCGAGC GTTTCAAGGA TGCGGAGGAC ATCCCGCGTC
       T  A  K     N  T  E  R     F  K  D     A  E  D     I  P  R  P      184
651  CCCCGTACTG GGGTGGGCTC CGTATTGTGC CCCTCGAGAT TGAGTTCTGG
       P  Y  W     G  G  L     R  I  V  P     L  E  I     E  F  W         200
701  CAGGGTCGTC CTTCTAGACT GCACGACCGT TTCGTGTACC GTCGTAAGAC
       Q  G  R  P     S  R  L     H  D  R     F  V  Y  R     R  K  T      217
751  GGAAAATGAT CCTTGGAAGG TCGTCCGTCT CGCCCCTTGA GGCATGTCGA
       E  N  D     P  W  K  V     V  R  L     A  P  *
801  GCGTAGAATT TGATTTTAGG TACGGTGAGA CCCGGTGTTCA GTTCGGTCG
851  ACCTGTTGTT GGCCATTTCT CGTCCGGTAG CATGTGGTCGT TATATGCGA
901  GACTCTCCGT AAAAAAAAAA AAAAA
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FIGURE 2 Nucleotide sequence and predicted amino acid sequence (single-letter amino acid code) of the *S. commune* pyridoxine (pyridoxamine) 5′-phosphate oxidase. The open reading frame (ORF) was defined by assigning the initiation codon ATG (at position 101). The translation stop code (TGA) is shown with an asterisk.

became reasonable to clone the gene of pyridoxine (pyridoxamine) 5′-phosphate oxidase from our library. A full-length cDNA clone from the *S. commune* cDNA library was isolated using a DNA hybridization probe amplified by polymerase chain reaction with degenerate primers, the design of which was based on conserved regions in sequences of pyridoxine (pyridoxamine) 5′-phosphate oxidase from other organisms. The cDNA clone[5] possessed an open reading frame encoding a polypetide of 229 amino acids with a calculated molecular weight of 26,359. The amino acid sequence given in **Figure 2** exhibits a striking degree of identity with the corresponding enzymes from *Saccharomyces cerevisiae* (76%), *Haemophilus influenzae* (51%), *Myxococcus xanthus* (48%), and *E. coli* (44%). The *S. commune* also contains a highly conserved region with a pattern of [LIVF] –E-F-W- [QH]-x(4)-R-[LIVM]-H-[DNE]-R, which, according to sequence analysis and previous studies in this laboratory, could be part of the active site of this enzyme. Yet another similar eukaryotic oxidase sequence, reported as Swiss-Prot: Q20939 in the EMBL/GenBank data files, is from the nematode *Caenorhabditis elegans*.

There is a mutual quenching of fluorescence of tryptophan upon binding of the coenzyme to the apoenzyme of the oxidase first isolated from the liver (Merrill et al. 1979). Using chemical modification techniques, it had been found that only one of the His residues is the base involved in catalysis and is protected by substract/product binding (Horiike et al. 1979). Based on these observations, it is reasonable to conclude that the highly conserved region contributes to the formation of the active site of this enzyme. ScanProsite sequence searches showed that all known sequences of pyridoxine (pyridoxamine) 5′-phosphate oxidase contain protein kinase C phosphorylation sites (5 in the *S. commune* sequence; positions 69–71, 73–75, 95–97, 168–170, and 172–174), casein kinase II phosphorylation sites (3 in the *S. commune* sequence; positions 64–67, 65–68, and 217–220), and tyrosine kinase phosphorylation sites (1 in the *S. commune* sequence; position 206–213). The *S. commune* sequence also has N-myristoylation sites (positions 29–34 and 94–99) near the amino terminus, as does that from *C. elegans*. These suggest that both *S. commune* and *C. elegans* enzymes may be N-terminal blocked. It had already been found that the pyridoxine (pyridoxamine) 5′-phosphate oxidase from rabbit livers is N-terminal blocked (McCormick et al. 1976). In another recent study, it was shown that the oxidase is developmentally regulated in the liver and brain; moreover, the mammalian oxidase cDNA will allow further investigations of the reasons for the absence of the oxidase in certain tumors (Ngo et al. 1998).

Finally it can be recalled that action of the broad specificity pyridoxal kinase from humans and most eukaryotes, followed by successive action of the oxidase, which has been shown competent to catalyze formation of PLP from numerous N-(5′-

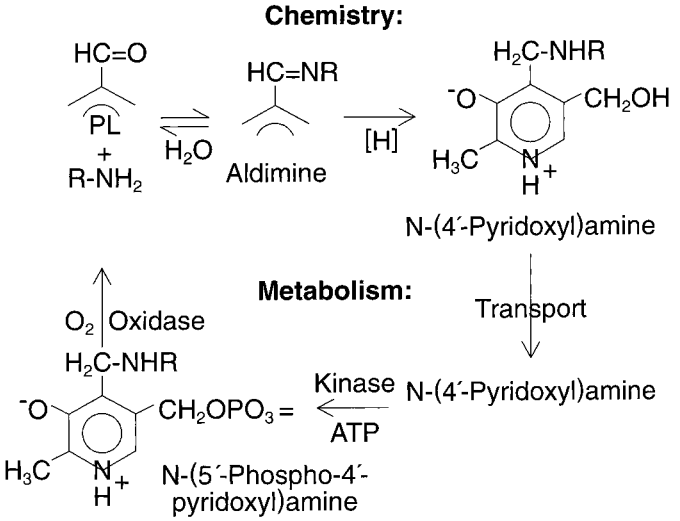

FIGURE 3 Consecutive action by pyridoxal kinase and pyridoxine (pyridoxamine) 5′-phosphate oxidase to liberate pyridoxal 5′-phosphate and a drug ($R\text{-}NH_2$) that has been covalently bound to pyridoxal for facilitated entry as the N-(4′-pyridoxyl)amine.

[5] The nucleotide sequence data reported in this paper have been submitted to the GenBank database under the accession number of AF 080236.

phospho-4′-pyridoxyl) amines as well as PMP and PNP, provide a model for transporter-enhanced delivery of bioactive compounds (Zhang and McCormick 1991). Generally for cells there is facilitated entry with relative specificity for water-soluble vitamins including the B-6 group (McCormick and Zhang 1993). Work on the uptake of B-6 by renal proximal tubular cells (Bowman and McCormick 1989), their brush-border membrane vesicles (Bowman et al. 1990), and binding proteins in the membranes (McCormick et al. 1991) has shown that all three natural non-phosphorylated forms of B-6 gain facilitated entry. Cells from the kidney and liver also import N-(4′-pyridoxyl)amines that was synthesized by condensing amines with pyridoxal and reducing the Schiff base product (Zhang and McCormick 1991, 1992a and b). The outline of such events, using pyridoxal and a drug bearing an amine function, to generate a stable, transportable compound that gains facilitated entry into cells where kinase and oxidase then liberate the original drug and PLP is illustrated in **Figure 3**. Some similar techniques may be applied in the ongoing search to effect better delivery of pharmacologic agents. In broader possibilities, a drug or other intracellular effector could be selectively piggybacked onto a transported solute such as a vitamin or other nutrient that gains facilitated entry to a cell and is, thereafter, metabolized to release the active compound.

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