

## MINI-REVIEW

# Vitamin B6 Deficiency, Genome Instability and Cancer

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### Abstract

Vitamin B6 functions as a coenzyme in >140 enzymatic reactions involved in the metabolism of amino acids, carbohydrates, neurotransmitters, and lipids. It comprises a group of three related 3-hydroxy-2-methyl-pyrimidine derivatives: pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM) and their phosphorylated derivatives [pyridoxal 5'-phosphate (PLP) and pyridoxamine 5'-phosphate (PMP)]. In the folate metabolism pathway, PLP is a cofactor for the mitochondrial and cytoplasmic isozymes of serine hydroxymethyltransferase (SHMT2 and SHMT1), the P-protein of the glycine cleavage system, cystathionine  $\beta$ -synthase (CBS) and  $\gamma$ -cystathionase, and betaine hydroxymethyltransferase (BHMT), all of which contribute to homocysteine metabolism either through folate-mediated one-carbon metabolism or the transsulfuration pathway. Folate cofactors carry and chemically activate single carbons for the synthesis of purines, thymidylate and methionine. So the evidence indicates that vitamin B6 plays an important role in maintenance of the genome, epigenetic stability and homocysteine metabolism. This article focuses on studies of strand breaks, micronuclei, or chromosomal aberrations regarding protective effects of vitamin B6, and probes whether it is folate-mediated one-carbon metabolism or the transsulfuration pathway for vitamin B6 which plays critical roles in prevention of cancer and cardiovascular disease.

**Keywords:** Vitamin B6 - folate - genome - epigenetics - homocysteine

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### Introduction: Pyridoxal 5'-phosphate (PLP) in Metabolism

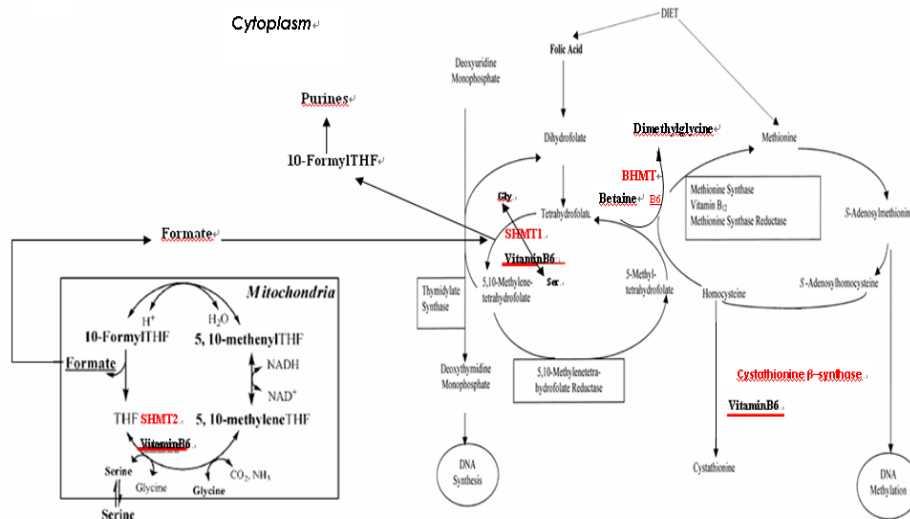
Vitamin B6, one of the B vitamins, is a water soluble, chemically quite distinct compound. It comprises a set of three different pyridine derivatives called pyridoxine (PN), pyridoxal (PL), and pyridoxamine (PM) (Ink, 1982; Hellmann, 2010). They differ in a variable group present at their 4- position with PN carrying a hydroxymethyl group, and PL and PM having an aldehyde and an aminomethyl group, respectively. Furthermore, all three B6 vitamers are phosphorylated by a kinase, which is a requirement for their role as cofactors in enzymatic reactions (Wu, 2011). While pyridoxamine-5'-phosphate (PMP) has been reported to function as a co-factor, it is pyridoxal 5'- phosphate (PLP) that is the biologically most active form (Lui et al., 1985; Gregory, 1997; Mann et al., 2011).

PLP plays a primary role acting as a cofactor for a large number of essential enzymes. These PLP-dependent enzymes catalyze more than 140 distinct enzymatic reactions including transaminations, aldol cleavages,  $\alpha$ -decarboxylations, racemizations,  $\beta$ - and  $\gamma$ - eliminations, and replacement reactions. For example, transaminases mediate the conversion of  $\alpha$ -ketoacids to amino acids and amino acid racemases produce D-amino acids from L-amino acids. Most of these reactions are related to amino acid biosynthesis and degradation. Another site

of action for the PLP-dependent enzymes is fatty acid metabolism. The enzyme  $\delta$ -6- desaturase catalyzes the synthesis of vital polyunsaturated fatty acids by the desaturation of linolic acid and  $\gamma$ -linolenic acid, respectively (Burns et al., 2005; Tanaka et al., 2005). Besides these roles, PLP also represents an important cofactor for the degradation of storage carbohydrates, such as glycogen. The PLP-dependent glycogen phosphorylase mediates the glycogen breakdown by the release of glucose from glycogen (Wagner, 2006). Furthermore, two PLP-dependent enzymes are involved in hemoglobin formation and chlorophyll biosynthesis. In these reactions the rate-limiting step is the primary biosynthesis of  $\delta$ -aminolevulinic acid. In mammals and birds  $\delta$ -aminolevulinic acid is synthesized by the action of  $\delta$ -aminolevulinic acid synthase and in plants and algae by the action of glutamate-1-semialdehyde 2, 1- aminomutase (Raschle et al., 2008).

Additionally, in plants the biosynthesis of the phytohormone ethylene is controlled by the synthesis of the precursor 1-aminocyclopropane-1-carboxylic acid from S-adenosylmethionine by PLP dependent 1-aminocyclopropane-1-carboxylate synthases (Lima et al., 2006; Kappes et al., 2011). This underlines the wide variety of chemical reactions that PLP-dependent enzymes promote in the organisms and shows again the importance of vitamin B6. The following section will give an overview

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**Figure 1. Methionine and Folate Metabolism - Locations of Vitamin B6, SHMT1, SHMT2, CBS, BHMT**

of the metabolic reactions in which PLP-dependent enzymes are significantly involved. Apart from its function as a cofactor for PLP-dependent enzymes, vitamin B6 is also thought to act directly as a protective agent against reactive oxygen species, such as singlet oxygen which will be discussed in a following section (Bitsch, 1993; Kannan et al., 2004; di Salvo et al., 2012).

### PLP- a Cofactor for the Mitochondrial and Cytoplasmic Isozymes of Serine Hydroxymethyltransferase (SHMT2 and SHMT1)

Folate-mediated one-carbon metabolism is compartmentalized in the mitochondria and cytoplasm of eukaryotic cells (Figure 1). In the cytoplasm, this metabolic network is required for the biosynthesis of purines, thymidylate, and the remethylation of homocysteine to form methionine. Serine is a major source of one-carbon units for this network through its reversible and tetrahydrofolate-dependent conversion to glycine and methylene tetrahydrofolate (methyleneTHF) is a metabolic cofactor that carries and activates single carbons for the synthesis of nucleotides and methionine catalyzed by serine hydroxymethyltransferase (SHMT), which is a PLP-dependent enzyme (Fox, 2008; Anderson et al., 2012). There are cytoplasmic and mitochondrial SHMT isozymes. SHMT1 encodes the cytoplasmic isozyme (SHMT1) and SHMT2 encodes the mitochondrial isozyme (SHMT2) (Garrow, 1993; Girgis, 1998; Stover, 1997; Hebbring et al., 2012). Mitochondrial one-carbon metabolism generates one-carbons from serine through the activity of SHMT2, and the one-carbon is oxidized and exported to the cytoplasm as formate, supporting cytoplasmic one-carbon metabolism (Herbig et al., 2002; Gutierrez et al., 2008). The SHMT1 enzyme generates methyleneTHF for thymidylate and methionine biosynthesis, but isotope tracer studies indicate that SHMT1 preferentially partitions methyleneTHF to thymidylate biosynthesis (MacFarlane et al., 2008; Anderson et al., 2009). The de novo thymidylate biosynthesis pathway requires three enzymes:

thymidylate synthase (TYMS), dihydrofolate reductase (DHFR), and SHMT1. MethyleneTHF generated by SHMT is the one-carbon donor for the TYMS catalyzed conversion of dUMP to dTMP generating dihydrofolate (DHF). DHFR catalyzes the NADPH-dependent reduction of DHF to regenerate THF for subsequent cycles of de novo thymidylate synthesis. Recently, the enzymes that constitute the thymidylate synthesis cycle were shown to undergo post-translational modification by the small ubiquitin-like modifier (SUMO) and nuclear translocation during S and G2/M phases (Anderson et al., 2007; Woeller et al., 2007). Although the synthesis of thymidylate in the nucleus has never been demonstrated, others have found folate cofactors present in liver nuclei (An et al., 2008), and multienzyme complexes containing ribonucleotide reductase and thymidylate synthase have been isolated from nuclear extracts (Noguchi et al., 1983; Ye et al., 2010). In Donald D. et al. study, intact nuclei are shown to catalyze the formation of dTMP from dUMP, which accounts for the results of stable isotope studies that indicate SHMT preferentially partitions methyleneTHF to thymidylate biosynthesis. Furthermore, both SHMT1 and SHMT2 are shown to contribute to nuclear de novo thymidylate biosynthesis (Donald, 2009).

### The Second PLP-dependent Enzyme - CBS

Cystathionine  $\beta$ -synthase (CBS) catalyzes the condensation of serine and homocysteine to form cystathionine and abnormality in CBS activity is manifested in two major clinical conditions, viz. hyperhomocysteinemia and homocystinuria (Yamasaki et al., 2012). Deficiency in the CBS activity is the most common cause of classical homocystinuria (HCU), an inherited human genetic disorder of sulfur amino acid metabolism biochemically characterized by very high levels of the toxic intermediate amino acid L-homocysteine (Hcy) (Pey et al., 2012). CBS catalyzes the  $\beta$ -replacement of the hydroxyl group of L-serine by the thiolate group of Hcy using PLP as cofactor, which is considered to be an independent risk factor for

arteriosclerosis (Meier et al., 2001). In addition to that, since homocysteine is vasculotoxic as well as neurotoxic, hyperhomocysteinemia predisposes to cardiovascular disorder (CVD) and cognitive dysfunction (Zhao et al., 2012; Liu et al., 2012). On the other hand, gross deficiency in CBS activity is associated with homocystinuria, an inborn recessive metabolic disorder (Yadav et al., 2012). The major pathologic abnormalities associated with homocystinuria include thromboembolism, ectopia lentis, osteoporosis, mental retardation (MR) and other neurological and psychiatric abnormalities (Liu et al., 2012; Boini et al., 2012). The neurological malfunctioning can be ascribed to the oxidation of excess homocysteine to homocysteic acid, which interacts with the N-methyl-D-aspartate receptor, causing excessive calcium influx and free radical production, thereby leading to neurotoxicity (Yadav et al., 2012). In addition, increased plasma homocysteine concentration has been postulated as a risk factor for cancer and even as a novel tumour marker (Yun et al., 2012). This increased risk can be attributed to the high prevalence of classical factors in these patients, such as hypertension, diabetes, and dyslipidemia, but most certainly (also) to factors resulting from the malignant disease and the applied selected therapy. For example, back in 1865 Trousseau described hypercoagulability and increasing risk of spontaneous coagulation in patients with cancer. Nowadays, it is established that breast, pancreas, and gastrointestinal cancers are associated with a higher incidence of thrombosis (Nadja et al., 2012). With more advanced stages of cancer there is lower overall survival rate (Renga, 2011), but, also a greater risk of venous thromboembolism, what can additionally influence on the survival of patients (Maclean et al., 2012).

### **The Third PLP-dependent Enzyme - BHMT**

Betaine-homocysteine methyltransferase (BHMT) catalyzes a key reaction at the convergence of the folate and the methionine cycles. BHMT is a PLP-dependent cytosolic enzyme that is highly expressed in the human liver, kidney and lens of the eye (Weisberg et al., 2003; Teng et al., 2012). It catalyzes one of two major homocysteine remethylation reactions, the transfer of a methyl group from betaine (N,N,N-trimethylglycine) to homocysteine, resulting in the formation of dimethylglycine and methionine. Betaine is the substrate for betaine-homocysteine methyltransferase (BHMT), acting as a methyl donor for methylating homocysteine (Fridman et al., 2012). Betaine also can be obtained from food or from choline metabolism (Clifford et al., 2012; Pawlik et al., 2011). Methionine is one of the essential dietary amino acids for humans and is the precursor for SAM (Košinek et al., 2012; Gibson et al., 2011). The tight interrelationship among these dietary methyl sources makes it important to assess them together when studying diet and its association with disease outcome. The other homocysteine remethylation reaction is catalyzed by methyltetrahydrofolate homocysteine methyltransferase (MTR) (Pellanda et al., 2012; Mostowska et al., 2011). BHMT is thought to account for up to half of the homocysteine remethylation capacity.

### **Vitamin B6 Deficiency, Genome Instability and Cancer**

Reduced dietary intake or low tissue/plasma levels of several vitamins B6 have been associated with higher risk for developing cancer (Sujol et al., 2011; Galluzzi et al., 2012; Lurie et al., 2012). Some studies on diet and cancer have disclosed a significant inverse correlation between serum PLP (and vitamin B6 intake) and different types of cancer (Hartman et al., 2001; Larsson et al., 2010; Wu et al., 2011; Galluzzi et al., 2012; Hellmann et al., 2010). There are several potential mechanisms by which vitamin B6 may influence carcinogenesis. First, B6-deficiency causes a decrease in the enzyme activity of SHMT and BHMT. This results in a lack of methylene groups for 5, 10-methylene-THF production. Consequently, methylation of deoxyuridylate to deoxythymidylate may be impaired resulting in misincorporation of uracil instead of thymidine into DNA (Bourquin et al., 2011; Kappes et al., 2011). As a consequence, a greater potential of chromosome strand breaks (Kamat et al., 2000; Romo et al., 2011) and/or an impaired DNA excision repair may exist (Ames, 2001; Bowling, 2011). Evidence of this has been reported (Ames, 1999). In addition, disruption of the above mentioned reactions may lead to imbalances in the methyl groups required for methylation processes, resulting in DNA hypomethylation. Altered DNA methylation has been observed in different types of tumors (Hansen et al., 1997; Cindy et al., 2005; Mann et al., 2011). The vitamin B6 connection to the immune system could be a mechanism by which low vitamin B6 status or intake also contributes to development of cancer. The two different PLP-dependent enzymes (CBS, cystathionine  $\gamma$ -lyase (CTH)) which are implicated in the transsulfuration pathway also generate cysteine, an important component of glutathione. Glutathione S-transferases and glutathione peroxidases are detoxifying agents of several carcinogenic compounds (Pey et al., 2012). PLP is also involved in steroid hormone action; consequently, PLP can be implicated in some types of steroid related cancer.

It is tempting to speculate that vitamin B6 inadequacy may be a factor in the aetiology of hormone-dependent cancer of the breast, uterus and prostate, and in hypertension; conditions where enhanced responsiveness of the target tissue to normal or even lower than normal levels of hormones may be important (Romo et al., 2011). Furthermore, one study found a steroid independent inhibition of *in vitro* breast cancer cell growth induced by PL and this was present in oestrogen-dependent and oestrogen-independent mammary carcinoma cell lines (Xu et al., 2008). Yu-Ching's findings suggest that higher intake of vitamin B6 is associated with a reduction in breast cancer risk, particularly ER-negative tumors (Yu et al., 2011). Many studies have shown a relation to special types of cancer. For example, in a large nested case-control study (included in the ATBC Cancer Prevention Study cohort) a statistically significant inverse dose-response relationship was found between plasma PLP levels and pancreatic cancer risk: the risk of subjects in the highest PLP tertile was half the risk of the subjects in the lowest tertile (OR = 0.48). Several case-control studies have

found that high vitamin B6 intake was associated with a decreased risk of gastric adenocarcinomas (Michaud et al., 2002; Ahn et al., 2008; Similä et al., 2009) and oral or pharyngeal cancer (Negri et al., 2000).

## Fruits, Vegetables and Vitamin B6

Fungi, plants, archae, and most eubacteria are able to synthesize vitamin B6, while most animal organisms, including humans, lack this ability and rely on the external supply of vitamin B6. Foods of animal origin contain mainly pyridoxamine (PM) and pyridoxal (PL), resulting in a bioavailability of approximately 75%, which approaches 100% in some foods. Vitamin B6 in foods of plant origin consists mainly of pyridoxine (PN) and the phosphorylated form – derivatives that have reduced bioavailability. Furthermore, a large proportion of the vitamin B6 content in foods of plant origin is glucosylated, which reduces the bioavailability further (Watanabe et al., 2004; Konings et al., 2006). For example, the bioavailability of pyridoxine glycosides (pyridoxine-50- b-D-glycosides) is approximately 50–58% that of free pyridoxine applied orally (Olsen et al., 2009). Furthermore, pyridoxine glycosides show an antagonistic effect on the metabolism of pyridoxine (Kalman et al., 2009). Therefore it can be assumed that persons with a dietary regimen that consists mainly (vegetarian diet) or exclusively (vegan diet) of plant foods are at risk of inadequate blood vitamin B6 concentrations. Some studies indicate that vegetarians have comparable vitamin B6 status to omnivorous study populations (García et al., 2007).

## Conclusion

Genome instability is strongly implicated in cancer, but a cause and-effect relationship remains to be proven. Although genome instability is very responsive to lowering with folic acid in most populations, other metabolically related B-vitamins, particularly vitamin B12 but also vitamin B6, have a role in preventing the elevation of tHcy and genome instability. Overall the nowadays studies indicate that vitamin B6 can be beneficial as a nutritional supplement, but can also be used as a pharmacological agent for cancer treatment.

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