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Modulation of gene expression by vitamin B_6

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The physiologically active form of vitamin B_6, pyridoxal 5′-phosphate (PLP), is known to function as a cofactor in many enzymic reactions in amino acid metabolism. Recent studies have shown that, apart from its role as a coenzyme, PLP acts as a modulator of steroid hormone receptor-mediated gene expression. Specifically, elevation of intracellular PLP leads to a decreased transcriptional response to glucocorticoid hormones, progesterone, androgens, and oestrogens. For example, the induction of cytosolic aspartate aminotransferase (cAspAT) in rat liver by hydrocortisone is suppressed by the administration of pyridoxine. The suppression of the cAspAT induction by pyridoxine is caused by a decrease in the expression of the cAspAT gene, which is brought about by inactivation of the binding activity of the glucocorticoid receptor to the glucocorticoid-responsive element in the regulatory region of the cAspAT gene. Vitamin B_6 has recently been found to modulate gene expression not only for steroid hormone-responsive or PLP-dependent enzymes but also for steroid- and PLP-unrelated proteins such as serum albumin. Albumin gene expression was found to be modulated by vitamin B_6 through a novel mechanism that involves inactivation of tissue-specific transcription factors, such as HNF-1 or C/EBP, by direct interaction with PLP in a similar manner to glucocorticoid receptor. Enhancement of albumin gene expression in the liver by an increased supply of amino acids can be explained by elevated binding of HNF-1 and C/EBP to their DNA-binding sites which, in turn, is caused by a decrease in the intracellular level of PLP by the increased amino acid supply. These findings that vitamin B_6 acts as a physiological modulator of gene expression add a new dimension to the hitherto recognized function of vitamin B_6 as a cofactor of enzyme action.

Vitamin B_6: Gene expression: Transcription factors: Steroid hormones: Albumin: Cancer

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Abbreviations cAspAT, cytosolic aspartate aminotransferase; GRE, glucocorticoid-responsive elements; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; PN, pyridoxine.

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Introduction

Vitamin B_6 is a water-soluble vitamin essential for normal growth, development and metabolism (Tryfiates, 1980; Merrill & Henderson, 1987). The physiologically active form of the vitamin, pyridoxal 5′-phosphate (PLP), is derived from inactive dietary precursors and functions as a cofactor in numerous enzyme reactions of amino acid metabolism (Merrill *et al*. 1984).

Apart from its role as a coenzyme, PLP is known to be an inhibitor of many enzymes that have binding sites for phosphate-containing substrates or effectors, including RNA polymerase (Venegas *et al*. 1973; Martial *et al*. 1975), reverse transcriptase (Basu *et al*. 1989), and DNA polymerase (Modak, 1976; Diffley, 1988). In all these cases, PLP is far more effective than other vitamers. It typically interacts with proteins by forming a Schiff base between its aldehyde group and primary amino groups, most commonly the ε-amino groups of lysine residues (Fisher *et al*. 1958).

The present review describes the recent emergence of vitamin B_6 as a modulator of gene expression through a novel mechanism that involves inactivation of tissue-specific transcription factors, in addition to its role in modulating gene expression in response to steroid hormones.

Vitamin B_6 and steroid hormone action

It is well known that vitamin B_6 affects enzyme induction by steroid hormones. Nishigori *et al*. (1978) first showed that treatment of partially purified progesterone receptor preparations with PLP could inhibit the binding of progesterone receptor to ATP-sepharose. At about the same time, Cake *et al.* (1978) showed that PLP inhibits binding of rat-liver glucocorticoid receptor to DNA-cellulose. Since then, several laboratories have established a correlation between vitamin B_6 and steroid hormone action. DiSorbo & Litwack (1981) reported that incubation of rat hepatoma in a pyridoxine (PN)-free medium resulted in a decrease in the intracellular level of PLP and significant enhancement of the induction of tyrosine aminotransferase. Similarly, the work of Majumder *et al*. (1983) showed that increased concentrations of vitamin B_6 inhibited glucocorticoid-induced casein mRNA accumulation in the mouse mammary gland. Allgood *et al*. (1990) demonstrated that vitamin B_6 modulates transcriptional activation by the human glucocorticoid receptor in HeLa cells. Allgood & Cidlowski (1992) subsequently showed that the modulatory effect of vitamin B_6 on transcription activation is not limited to the glucocorticoid receptor but extends to multiple members of the steroid hormone receptor superfamily. They further reported that modulation of vitamin B_6 of glucocorticoid receptor-mediated gene expression requires transcription factor NF1 in addition to the glucocorticoid receptor (Allgood *et al*. 1993). These results indicate that increased intracellular PLP leads to decreased transcriptional responses to glucocorticoids, progesterone, androgens and oestrogens. Conversely, cells in a vitamin B_6-deficient state exhibit enhanced responsiveness to steroid hormones. These studies have been reviewed by Tully *et al.* (1994).

Vitamin B_6 and gene expression of liver enzymes

The evidence for an interaction between PLP and steroid hormone receptors, described in the preceding section, has been mainly obtained with the use of subcellular extracts or cultured cells. However, an indication of possible interaction between vitamin B_6 and steroid hormones in the whole animal has existed for some time (Bender, 1987, 1994). For example, cytosolic aspartate aminotransferase (cAspAT) in rat liver is a PLP-dependent enzyme and the activity of

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the enzyme is induced by the administration of glucocorticoid hormones. Kondo & Okada (1985) found that the induction of the enzyme in the liver of adrenalectomized vitamin B_6-deficient rats by hydrocortisone was suppressed by the administration of PN.

We recently found that the level of cAspAT mRNA in the liver of vitamin B_6-deficient rats was several-fold higher than that of the control rats (Oka *et al*. 1995*a*). The administration of hydrocortisone induced expression of the hepatic cAspAT mRNA and the induction was suppressed by the simultaneous administration of PN. Since the 5′ regulatory region of the rat cAspAT gene contains several sequences showing homology to glucocorticoid-responsive elements (GRE), we synthesized an oligonucleotide probe of GRE sequence and assayed the binding activity of nuclear extract to the oligonucleotide by gel mobility shift analysis. We found that the binding activity of nuclear extract prepared from the liver of vitamin B_6-deficient rats was far greater than that of the control rats, indicating that the DNA-binding activity of the glucocorticoid receptor was enhanced by vitamin B_6 deficiency. We further found that preincubation of the nuclear extract with PLP brought about a rapid and extensive decrease in the binding of the extract to GRE. Analogues of PLP, such as pyridoxamine 5′-phosphate (PMP), pyridoxal, pyridoxamine or PN, did not show an inhibitory effect. These observations suggest that PLP modulates cAspAT gene expression by inactivating the binding of glucocorticoid receptor to GRE.

Glycogen phosphorylase is another PLP-dependent enzyme, which catalyzes the first step in the intracellular degradation of glycogen. We observed that the level of phosphorylase mRNA was also increased several-fold in the liver of vitamin B_6-deficient rats, compared with vitamin B_6-supplemented controls (Oka *et al*. 1994). Presumably, vitamin B_6 acts in the same way as in the case of cAspAT. However, the effect of vitamin B_6 deficiency on phosphorylase was tissue-specific; the level of phosphorylase mRNA in the skeletal muscle of vitamin B_6-deficient rats was reduced, to 40 % of that in the control rats, rather than increased as in the liver.

In the course of the study on the effect of vitamin B_6 deficiency on the expression of liver enzymes, we made an unexpected observation. cAspAT and phosphorylase, two enzymes so far described, are PLP-dependent enzymes and one may rationalize the involvement of vitamin B_6 in the control of expression of their genes. However, examination of additional liver enzymes revealed that gene expression of several enzymes, not related to PLP or steroid hormones, were also influenced by vitamin B_6 nutritional status. Thus, mRNA levels of apolipoprotein A-1, phenylalanine hydroxylase, glyceraldehyde-3-phosphate dehydrogenase and β-actin were elevated in the liver of vitamin B_6-deficient rats (Oka *et al*. 1993). Additionally, the activities of RNA polymerase I and II were found to be increased in vitamin B_6 deficiency. The latter finding indicates that the earlier demonstration *in vitro* of PLP as an inhibitor of RNA polymerase (Venegas *et al*. 1973; Martial *et al*. 1975) may have some physiological significance. The enhanced expression of several vitamin B_6-independent enzymes in the liver of vitamin B_6-deficient rats might be explained, at least in part, by the elevation of RNA polymerase activity.

Vitamin B_6 and expression of the albumin gene

Serum albumin represents the most abundant protein synthesized in and secreted by the liver. Among the vitamin B_6-independent enzymes and proteins whose gene expression is enhanced by vitamin B_6 deficiency, we noted that the level of albumin mRNA was increased 7-fold over the control level (Oka *et al*. 1995*b*). The magnitude of the increase in albumin mRNA was far greater than can be explained in terms of increased RNA polymerase activity.

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Studies of transcription of the rat albumin gene have shown that the 170-nucleotide region immediately upstream of the transcription initiation site is sufficient for tissue-specific expression of the gene. Several *cis*-acting elements have been identified in this region that interact with various transcription factors including HNF-1, C/EBP, CTF/NF1 and NFY (Cereghini *et al*. 1987; Marie *et al*. 1989) (Fig. 1). However, there is a hierarchy of importance of the various factors for albumin gene expression; the HNF-1 (–45 to –62) and C/EBP-binding sites (–136 to –160) activate transcription more strongly than the other sites (Marie *et al*. 1989). We synthesized two oligonucleotides, which interact with HNF-1 and C/EBP respectively, and determined the binding activities of liver nuclear extracts to each of these oligonucleotides by mobility-shift analysis. We found that the activity of the extract prepared from liver of vitamin B_6-deficient rats was greater than that of the controls. As the concentrations of C/EBP in nuclear extracts from control and vitamin-deficient rats, estimated by Western-blot analysis, were essentially the same, the lower binding activity of the extract from control liver is probably due to inactivation of tissue-specific factors by PLP and/or its analogues. We therefore examined the effect of PLP and other vitamin B_6 vitamers on the binding activity of nuclear extract *in vitro* and found that only PLP effectively inhibited the binding. These observations are analogous to the inactivation of glucocorticoid receptor by PLP, described above, and indicate that vitamin B_6 modulates albumin gene expression through a novel mechanism that involves inactivation of tissue-specific transcription factors by direct interaction with PLP (Fig. 2).

In order to elucidate the molecular mechanism whereby DNA-binding activity of tissue-specific transcription factors is inhibited by PLP, we recently produced recombinant HNF-1 in *Escherichia coli* and determined the site of attachment of PLP *in vitro*. Determination of amino acid sequence of PLP-containing peptide revealed that PLP was bound to lysine 197 of HNF-1 molecule (T Oka, unpublished results; Fig. 3). Inasmuch as lysine 197 lies in the homeodomain of HNF-1, PLP binding to this lysine residue would render the HNF-1 molecule less accessible to the HNF-1 binding site of the albumin gene.

Expression of the albumin gene and amino acid and protein nutrition

As noted above, serum albumin represents the most abundant protein synthesized in the liver. It has long been recognized that the protein-nutritional status of animals affects the synthesis of albumin. Chiku *et al*. (1993) showed that depletion of amino acid supply to rats during total parenteral nutrition did not alter the fractional synthesis rate of liver domestic proteins but decreased the synthesis rate of serum proteins, particularly albumin. However, little is known about the molecular mechanism whereby the levels of amino acid supply to animals differentially affects the synthetic rates of domestic proteins and serum proteins in the liver.

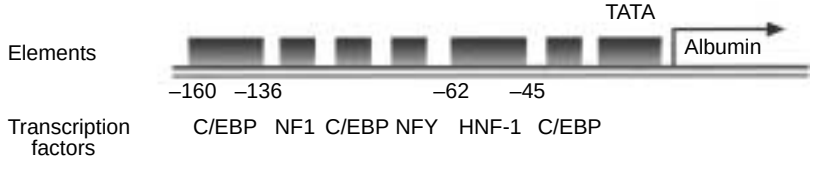

Fig. 1. Schematic representation of the transcriptional factors interacting with the regulatory region of the albumin gene. The HNF-1-(–45 to –62) and C/EBP-binding sites (–136 to –160) activate transcription more strongly than the other sites (Marie *et al*. 1989).

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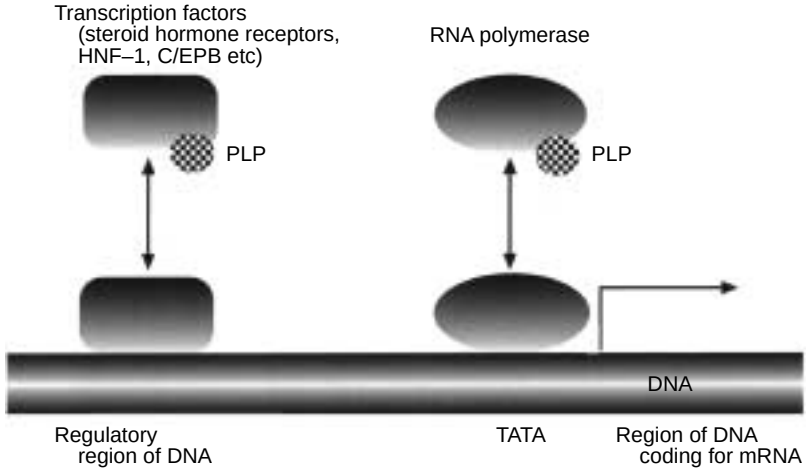

Fig. 2. Diagram of binding of transcription factors and RNA polymerase to a regulatory region and a TATA site of DNA respectively. DNA-binding activities are inhibited by interaction of pyridoxal 5′-phosphate (PLP) to transcription factors and RNA polymerase.

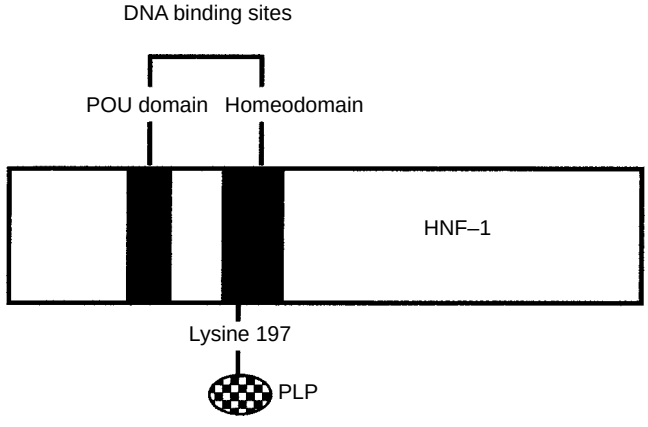

Fig. 3. Binding of pyridoxal 5′-phosphate (PLP) to transcription factor, HNF-1. PLP-binding to lysine-197 is in the homeodomain of HNF-1.

In our recent study (Oka *et al*. 1997), rats were nourished by infusion of total parenteral nutrition solutions containing 0 or 33 g amino acids/kg for 7 d. The level of albumin mRNA in the liver of amino acid-infused rats was found to be several-fold higher than that in the liver of amino acid-depleted rats. Since the expression of the albumin gene is regulated by tissue-specific transcription factors, HNF-1 and C/EBP, we determined the binding activities of nuclear extracts to the HNF-1- and C/EBP-binding sites by mobility-shift analysis and found that the activity of the extract prepared from livers of amino acid-infused rats was greater than that of amino acid-depleted rats. In view of the involvement of vitamin B_6 in the modulation of albumin gene expression, we determined the intracellular concentrations of vitamin B_6 derivatives and found that the PLP concentration in the liver of amino acid-infused rats was decreased

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to almost a half that of amino acid-depleted rats while PMP concentration was increased proportionally. These observations suggest that a decrease in intracellular PLP, in turn, relieves inactivation of tissue-specific transcription factors for the expression of the albumin gene.

The question as to how amino-acid infusion decreases hepatic PLP concentration may be raised. Amino acids, transported into hepatic cells, will undergo transamination reactions catalyzed by aminotransferases. Since PLP is the coenzyme of all the aminotransferase and enzyme-bound PLP is converted to PMP during transamination, the continuous influx of amino acids may decrease PLP concentration and elevate PMP concentration in the steady state (Fig. 4).

Vitamin B_6 and platelet aggregation

PLP has been shown to inhibit *in vitro* platelet aggregation induced by ADP, collagen, thrombin, adrenaline and arachidonic acid (Kloczowia & Freinberg, 1980; Chang & Mak, 1999). It is well known that platelet aggregation is mediated by a common mechanism that involves the binding of fibrinogen or other adhesive proteins to GPIIb/IIIa complexes of platelets (Nurden & Phillip, 1990). GPIIb/IIIa is the major receptor protein on platelet membranes and is responsible for aggregation. The final step of all platelet-aggregating agents is the functional expression of GPIIb/IIIa on the platelet surface, which ligates fibrinogen to link platelets together as part of the aggregation process (Verstraete & Zoldhelyi, 1995).

Glycoprotein IIb (GPIIb) is the α-subunit of the platelet membrane receptor GPIIb/IIIa, which plays a major role in platelet aggregation. Chang *et al*. (1999) analysed the molecular mechanism of vitamin B_6 on anti-aggregation of platelet by analysing the expression of GPIIb promoter-driven reporter gene. The GPIIb promoter region was amplified by polymerase chain reaction, cloned into pBLCAT3 to drive the CAT reporter gene and transfected into human erythroleukaemia cells. Transient expression of the GPIIb promoter was determined after transfected cells were treated with 1 μM PN, pyridoxal, PLP or 4-deoxypyridoxine. GPIIb promoter activity was down-regulated to 54, 35 and 63 % in the presence of PN, pyridoxal and PLP respectively as compared with an untreated control. However, no adverse effect on GPIIb promoter was detected by 4-deoxypyridoxine, which is an antagonist of vitamin B_6. The down-regulatory effect of vitamin B_6 on GPIIb promoter activity may lead to a reduction of GPIIb protein expression and thus be detrimental to platelet aggregation.

These observations are analogous to the inactivation of HNF1 and C/EBP by PLP, described above, and indicate that vitamin B_6 modulates GPIIb gene expression through a novel

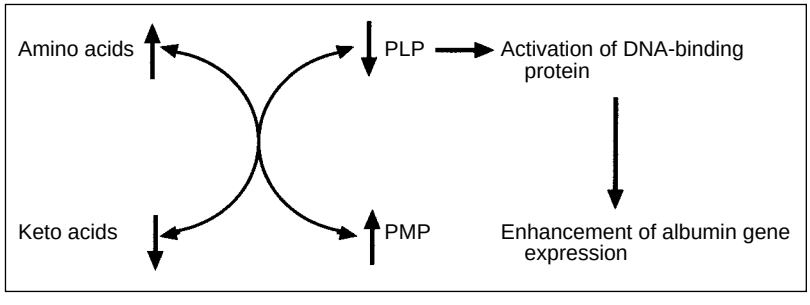

Fig. 4. Proposed regulatory mechanism of albumin gene expression by amino acids. PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate.

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mechanism that involves inactivation of tissue-specific transcription factors by direct interaction with PLP.

Vitamin B_6 and gene expression of c-fos in nerve cells

Alterations in neuronal proto-oncogene expression have been reported in conditions such as brain injury, receptive stimulation, or seizures induced by metrazol or kindling (Dragunow & Robertson, 1987, 1988). For example, c-fos expression is increased after seizures induced by metrazol (Morgan *et al*. 1987).

It is known that vitamin B_6 is involved in nerve cell function and vitamin B_6 antagonists cause seizures in various mammals (Makino & Matsuda, 1960). Mizuno *et al*. (1989) examined the effect of vitamin B_6 antagonists on the expression of the c-fos gene in the brain. They observed that only 4-deoxypyridoxine among various vitamin B_6 antagonists was effective in increasing the level of c-fos mRNA and c-fos protein in nerve cells; c-fos mRNA increased slightly at 10 min after deoxypyridoxine administration, was maximal at 60 min and then returned to the basal level at 120 min. The clinical manifestation of seizures corresponded well with the increase in c-fos mRNA level in brain around 60 min after deoxypyridoxine administration. Treated mice were observed to behave normally at 120 min when c-fos mRNA had returned to the basal level. Administration of deoxypyridoxine causes a vitamin B_6-deficient state in mice; these observations also indicate that vitamin B_6 modulates c-fos gene expression through a novel mechanism.

Vitamin B_6 and cancer

The level of plasma vitamin B_6 in cancer patients is lower than that of healthy subjects (Potera *et al*. 1977; Chrisley & Hendricks, 1986), suggesting that administration of vitamin B_6 to cancer patients may be therapeutically useful. The growth of rat hepatoma Fu5–5 cells and human malignant melanoma NEL cells was inhibited on culture in a medium supplemented with 5 mM and 0·5 mM PN respectively (DiSorbo & Litwack, 1982; DiSorbo & Nathanson, 1983).

We recently found that the growth of human hepatoma HepG2 cells was completely inhibited in medium supplemented with PN in the millimolar range. The growth inhibition of HepG2 cells was accompanied by a marked inhibition of gene expression, protein synthesis and secretion of serum proteins, particularly albumin. Pyridoxal was as effective as PN, but pyridoxamine showed no inhibitory action (Molina *et al*. 1997). The electron-microscopic examination of PN-treated HepG2 cells revealed a smoothing of nuclear membrane, a decrease in the number of nucleoli, and the appearance of aggregated heterochromatin structures. These morphological features were compatible with depressed transcriptional activity in the PN-treated cells. We also found that the growth of MH-134 hepatoma cells, transplanted into C3H/He mice, was significantly retarded by the administration of large doses of PN. The expression of oncogenes, such as c-fos and c-myc, was considerably reduced in the tumours, probably by a similar mechanism to the inhibition of albumin gene expression by vitamin B_6 (T Oka, unpublished results).

The anti-tumour effect of vitamin B_6 is not limited to liver cancer alone; recent studies have shown that dietary supplementation of vitamin B_6 markedly suppresses azoxymethane-induced colon carcinogenesis in mice (Komatsu *et al*. 2001). Application of the supraphysiological doses of vitamin B_6 as an antineoplastic therapy is a promising area for future research.

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Conclusions

Vitamin B_6 has long been recognized as a cofactor for many enzymes, especially those involved in amino acid metabolism. Apart from its role as coenzyme, recent studies are unveiling a new role of vitamin B_6 as a modulator of gene expression. Vitamin B_6 modulates gene expression by interaction with not only steroid hormone receptors but also with many tissue-specific transcription factors such as HNF-1 and C/EBP.

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