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Effect of vitamin B_6 deficiency on the expression of glycogen phosphorylase mRNA in rat liver and skeletal muscle

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Abstract. The effect of vitamin B_6 deficiency on the expression of glycogen phosphorylase mRNA in rat liver and skeletal muscle was investigated. The level of phosphorylase mRNA in the muscle of vitamin B_6-deficient rats was reduced to 40% of that in the control rats. By contrast, the phosphorylase mRNA level was increased 5-fold in the liver of the deficient animals. It was also found that the expression of the β-actin gene, generally regarded as a 'housekeeping' gene, was unaffected by B_6 deficiency in the muscle but was enhanced in the liver of the deficient animals. These observations suggest that vitamin B_6 may modulate the transcriptional activation of the phosphorylase gene in a tissue-specific manner.
Key words. Vitamin B_6; glycogen phosphorylase mRNA; rat liver and muscle.

Glycogen phosphorylase (EC 2.4.1.1) catalyzes the first step in the intracellular degradation of glycogen. Extensive investigation has defined the allosteric effectors and covalent phosphorylation involved in the regulation of phosphorylase activity[1,2]. Phosphorylase exists in immunologically distinct isozyme forms in muscle and liver[3,4], and phosphorylase levels are regulated in both tissues.

Phosphorylase contains pyridoxal phosphate as a cofactor, covalently bound via a Schiff base to an active site lysine[5,6]. We recently found that vitamin B_6 deficiency in rats differentially affected phosphorylase in liver and skeletal muscle; the deficiency caused significant decreases in the activity and amount of phosphorylase in muscle while the enzyme activity in liver was virtually unchanged[7]. We have now examined the effect of vitamin B_6 deficiency on the expression of phosphorylase mRNA in rat liver and skeletal muscle.

Material and methods

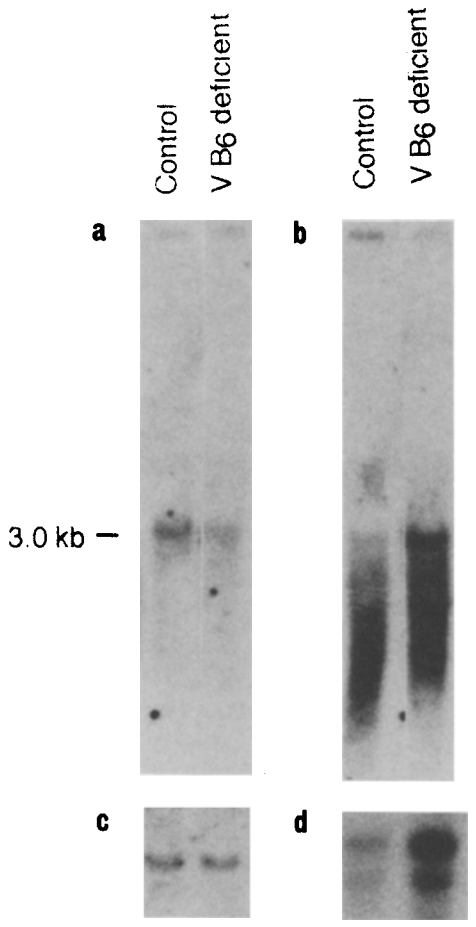
Animals. Male weanling rats of the Wistar strain were given a 70% casein diet with or without pyridoxine ad libitum for 4 weeks. The composition of the diet was as previously described[8].

Northern blot analysis. Total cellular RNA was isolated from pooled livers and muscles (5 rats each in the control and vitamin B_6-deficient groups) by the guanidine-isothiocyanate method[9] and poly(A)$^+$ RNA was fractionated using an oligo(dT)-cellulose column (Pharmacia, Sweden). Poly(A)$^+$ RNA (10 µg) was electrophoresed in a 1.5% formaldehyde-agarose gel, blotted on to a nitrocellulose membrane and hybridized to the muscle-type glycogen phosphorylase cDNA (kindly provided by Dr. S. Osawa, University of Massachusetts) as described previously[10]. The cDNA probe was labeled using a Random Primed Labeling Kit (Takara Shuzo Co., Japan) according to the protocol provided. The blot was washed twice in 2 × SSC, 0.1% SDS at 65 °C and once in 1 × SSC, 0.1% SDS at 65 °C, and then an autoradiograph made using Kodak X-Omat film at −70 °C.

Results and discussion

Northern blot analysis of skeletal muscle and liver glycogen phosphorylase mRNA is shown in the figure. The size of the mRNA agreed with the published values of 3.2 and 3.1 kb for muscle and liver phosphorylase mRNA, respectively[11]. Since the sequence of rat muscle mRNA is 74% homologous with the liver mRNA[11], both muscle and liver mRNAs were probed with the muscle phosphorylase cDNA. As shown in the figure (a), the level of phosphorylase mRNA in the muscle of vitamin B_6-deficient rats was reduced to 40% (estimated by scanning densitometry) of that in the control rats. By contrast, the phosphorylase mRNA level was increased 5-fold in the liver of the deficient animals (fig., b).

We recently reported that the activity and amount of phosphorylase in the muscle of vitamin B_6-deficient rats were decreased to about 40% of their levels in control rats, while the activity of the enzyme in the liver was virtually unchanged by vitamin B_6 deficiency[7]. Assuming that the rate of synthesis of any protein is in general proportional to the quantity of its corresponding mRNA[12], the decrease in the amount of phosphorylase in the muscle of vitamin B_6-deficient rats may be explained as a consequence of the decreased abundance of



Northen blot analysis of glycogen phosphorylase and β-actin mRNAs from control and vitamin B_6-deficient rats. *a* Muscle phosphorylase mRNA; *b* liver phosphorylase mRNA; *c* muscle β-actin mRNA; and *d* liver β-actin mRNA. 10 µg of poly(A)$^+$ RNA from 5 rats was applied in each lane. The autoradiographs were densitometrically scanned in order to determine mRNA levels quantitatively. The experiment shown is representative of 4 independent experiments which gave essentially the same results.

phosphorylase mRNA. It is of interest to note that a denervation-induced muscular atrophy in mice was also accompanied by decreases in the abundance of both phosphorylase and phosphorylase mRNA in the muscle[13]. As regards the mechanism of modulation of phosphorylase mRNA level by vitamin B_6, it is not possible to discriminate between decreased synthesis and/or accelerated degradation of the mRNA.

In contrast with the situation in the muscle, the phosphorylase mRNA level in the liver was increased in vitamin B_6 deficiency. If the rate of synthesis of phosphorylase is proportional to the amount of phosphorylase mRNA, one would expect an increase in the enzyme level in the vitamin-deficient liver. However, our previous work indicated that the phosphorylase level in the liver of the deficient rats was essentially the same as that in the liver of control rats[7]. This could occur if not only the rate of synthesis but also the rate of degradation of phosphorylase in the liver were increased in vitamin B_6 deficiency. We previously observed an analogous situation with cytosolic aspartate aminotransferase, another pyridoxal phosphate-dependent enzyme in the liver: under conditions of vitamin B_6 deficiency, the level of the enzyme remained the same, whereas both the rate of synthesis and the rate of degradation of the enzyme were increased[14]. The increased rate of degradation of cytosolic aspartate aminotransferase was recently shown to be due to an accelerated sequestration of the enzyme into lysosomes[15]. It is very likely that the lysosomal degradation of phosphorylase is similarly enhanced in the liver of vitamin B_6-deficient rats.

The expression of the β-actin gene is generally thought to be constant under various physiological conditions, so that it can be used as an internal control. We observed that the expression of β-actin mRNA was essentially the same in the muscles of control and vitamin B_6-deficient animals (fig., c) but the mRNA level was increased three-fold in the liver of the deficient animals (fig., d). We recently observed an enhancement of the activities of DNA-dependent RNA polymerases I and II in the liver of vitamin B_6-deficient rats, which suggested that alterations in the intracellular concentrations of pyridoxal phosphate influence gene expression in the liver, at least in part, by modulating the activity of RNA polymerase[16]. One should note, however, that the magnitude of increase in the level of phosphorylase mRNA was clearly greater than that of β-actin mRNA in the liver of vitamin B_6-deficient rats. Recent studies have shown that vitamin B_6 modulates transcriptional activation by way of a number of members of the steroid hormone receptor superfamily, including the glucocorticoid receptor[17,18]. Whether or not the tissue-specific action of vitamin B_6 on phosphorylase gene expression involves a similar mechanism remains to be established.

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