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Vitamin B_6 deficiency causes activation of RNA polymerase and general enhancement of gene expression in rat liver

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The effect of vitamin B_6 deficiency on the activity of RNA polymerase and expression of several mRNAs in rat liver was investigated. The activities of RNA polymerase I and II in the liver of vitamin B_6-deficient rats were found to be higher than the control rats by 30%. The expression of several mRNAs, including mRNAs for β-actin and glyceraldehyde-3-phosphate dehydrogenase, and the content of poly(A)$^+$ RNA were also increased in vitamin deficiency. These observations suggest that vitamin B_6 influences gene expression in the liver, at least in part, by modulating the activity of RNA polymerase.

Vitamin B_6; RNA polymerase; Gene expression; Rat liver

1. INTRODUCTION

Pyridoxal 5′-phosphate (PLP) is the coenzyme of several enzymes of amino acid metabolism [1]; it is also an essential constituent of glycogen phosphorylase [2,3]. In these enzymes, PLP is covalently bound via a Schiff base to an active site lysine residue [4]. Apart from its role as the coenzyme, PLP has proven to be an effective inhibitor of many enzymes which have binding sites for phosphate-containing substrates or effectors including RNA polymerases [5,6], reverse transcriptases [7], *E. coli* DNA polymerase I [8] and animal cell DNA polymerases [9,10]. In all these studies, PLP has been used as a molecular probe to identify specific amino acid residues required for the catalytic activities in vitro. Very little is known, however, on the influence of intracellular vitamin B_6 concentration on the activities of these enzymes in vivo.

We have examined the activity of RNA polymerase and expression of several mRNAs in the liver of vitamin B_6-deficient rats. Results from the present study suggest that alterations in PLP concentration influence gene expression, at least in part, by modulating the activity of RNA polymerase.

2. MATERIALS AND METHODS

2.1. *Animals and chemicals*

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 described previously [11]. [^{3}H]UTP (43 Ci/mmol) and [^{32}P]dCTP (3,000 Ci/mmol) were purchased from ICN Biochemicals Inc., CA, USA. Random Primed Labeling Kit was purchased from Takara Shuzo Co., Tokyo, Japan. Oligo-dT was synthesized in our laboratory using a DNA synthesizer. Sources of cDNA probes were as follows: apolipoprotein A-I, obtained from Dr. J.L. Breslow (Harvard Medical School, Boston, MA, USA); glucocorticoid receptor, obtained from Dr. R.M. Evans (Salk Institute, La Jolla, CA, USA); phenylalanine hydroxylase and glyceraldehyde-3-phosphate dehydrogenase, obtained from American Tissue Culture Collection (Rockville, MD, USA); and β-actin, obtained from Dr. D.W. Cleaveland (University of California, San Francisco, CA, USA).

2.2 *Slot blot analysis*

Total cellular RNA was isolated from pooled livers (5 rats each in the control and vitamin B_6-deficient groups) by the guanidine thiocyanate method [12] and applied with a manifold onto a nitrocellulose membrane and hybridized to various cDNAs as described previously [13]. The probes were labeled with [^{32}P]dCTP according to the Random Primed Labeling Kit protocol. 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 exposed to Kodak X-Omat film at −70°C. The densities of autoradiographic bands was quantitated using an LKB Ultro Scan Laser Densitometer.

2.3 *RNA synthesis in isolated nuclei*

Preparation of nuclei from livers and assay of RNA synthesis in isolated nuclei were described previously [14]. The relative activities of RNA polymerase I, II and III were estimated by analyzing the pattern of inhibition of RNA synthesis by α-amanitin [15].

3. RESULTS

Hybridizability of the total liver RNA with several cDNA probes was analyzed by slot blotting (Fig. 1). Validity of the slot blotting as a measure of the hybridization signal from each mRNA was verified by North-

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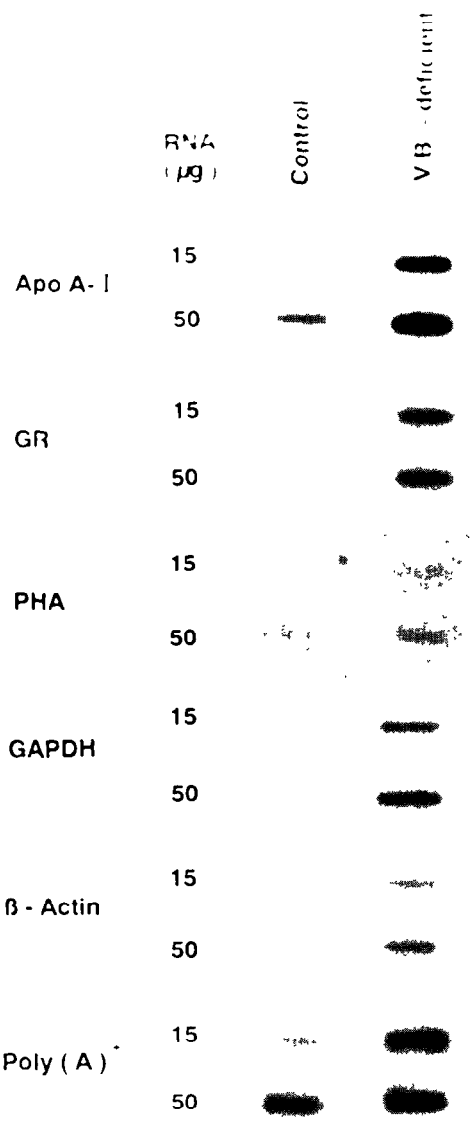

Fig. 1. Slot blot analysis of apolipoprotein A-I (ApoA-I), glucocorticoid receptor (GR), phenylalanine hydroxylase (PAH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-actin mRNAs and poly(A)$^+$ RNA from the livers of rats fed control or vitamin B_6-deficient diets for 4 weeks. Two different quantities (15 and 50 μg) of total cellular RNA from pooled livers (5 rats each in control and vitamin-deficient groups) were applied and probed with various cDNAs.

ern blotting (data not shown). It appears that the levels of all mRNAs tested in the liver of vitamin B_6-deficient rats are higher than those in the control liver. Semiquantitative estimation by densitometric scanning indicated that the levels of mRNAs for apolipoprotein A-1, glucocorticoid receptor, phenylalanine hydroxylase, glyceraldehyde-3-phosphate dehydrogenase and β-actin in the liver of vitamin B_6-deficient rats were 7-, 4-, 3-, 4- and 3-fold higher than those in the liver of control rats, respectively. Furthermore, the slot blotting with an oligo(dT) probe indicated that the relative content of poly(A)$^+$ RNA in the total cellular RNA from the liver of vitamin-deficient rats was twice that of the control rats.

We next examined the influence of vitamin B_6 deficiency on RNA synthetic activity in the liver. The relative activities of various RNA polymerases were estimated by differential inhibition of RNA synthesis by α-amanitin [15]. The synthetic activities of rRNA (RNA polymerase I) and mRNA (RNA polymerase II) in nuclei isolated from the liver of vitamin B_6-deficient rats were higher than the control rats by 30% while the synthesis of tRNA (RNA polymerase III) was not influenced by vitamin B_6 deficiency (Fig. 2).

4. DISCUSSION

As described in Section 1, PLP has been shown to be a potent inhibitor of RNA polymerases and DNA polymerases from a wide variety of sources, ranging from viruses to eukaryotes, when assayed in vitro. However, the possible influence of vitamin B_6 on the activities of these enzymes in vivo has remained unknown. The present study demonstrated an enhancement of the activities of DNA-dependent RNA polymerases I and II in the liver of vitamin B_6-deficient rats, suggesting that PLP inhibits RNA polymerase not only in vitro but also in vivo. It was also found that the expression of several mRNAs was induced in the liver of vitamin B_6-deficient rats. Since we observed an increase in the relative content of poly(A)$^+$ RNA in the deficient liver, the effect of vitamin B_6 deficiency is probably not limited to the expression of several mRNAs, presently examined, but rather represents general activation of mRNA synthesis. Although the expression of individual mRNAs may be controlled by various transcriptional factors, the present results suggest that alterations in the intracellular concentration of PLP influence gene expression in the liver, at least in part, by modulating the activity of RNA polymerase.

Recent studies have shown that vitamin B_6 modulates transcriptional activation by glucocorticoid receptor [16] as well as other members of the steroid hormone receptor superfamily [17]. Specifically, the level of the steroid hormone-mediated gene expression is reduced under conditions of vitamin elevation, and enhanced in vitamin deficiency. The expression of the mRNAs, whose levels were presently analyzed by slot blotting, is not generally considered as steroid hormone-dependent. The expression of β-actin and glyceraldehyde-3-phosphate dehydrogenase mRNAs, among others, is generally thought to be constant under various physiological conditions, thus being used as internal controls. Since the levels of these mRNAs were clearly elevated in the liver of vitamin B_6-deficient rats, the modulatory effects of the vitamin are not limited to steroid hormone-mediated gene expression but may involve hormone-independent gene expression as well.

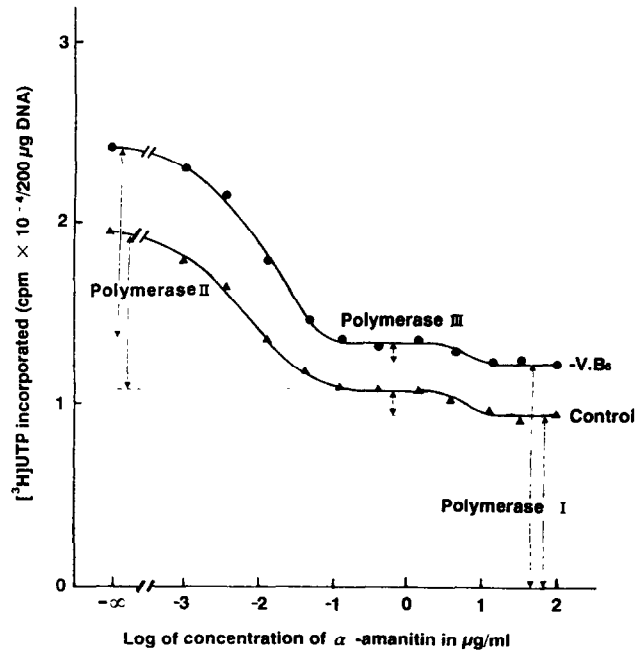

Fig. 2 RNA synthesis in isolated nuclei. RNA synthesis was carried out with nuclei isolated from control (▲) and vitamin B_6-deficient (●) rat livers. Varying concentrations of α-amanitin were included in each reaction mixtures and the activities of RNA polymerase I, II and III were estimated as in ref. 15.

Regarding glucocorticoid receptor-dependent gene expression, Allgood et al. [16] reported that vitamin B_6 status did not affect the level of glucocorticoid receptor mRNA. However, we observed that the level of glucocorticoid receptor mRNA was elevated in vitamin deficiency. The discrepancy may be due to difference in cell types; they used cultured HeLa S_3 cells and we used rat liver in the present study. Another possible source of difference is that they estimated the level of glucocorticoid receptor mRNA after normalization with the level of β-actin mRNA, assuming that the level of β-actin mRNA was constant under variable vitamin B_6 status. We observed presently that this assumption did not hold true in rat liver; the levels of glucocorticoid receptor and β-actin mRNAs were elevated to approximately the same extent in vitamin B_6 deficiency.

The data presented here demonstrate that vitamin B_6 influences not only steroid hormone receptor-dependent gene expression but also hormone-independent gene expression by modulating the activity of RNA polymerase.

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