Plant Physiology and Biochemistry 66 (2013) 63–67

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Short communication

Effect of abiotic stress on the abundance of different vitamin B_6 vitamers in tobacco plants

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ARTICLE INFO

Article history:
Received 8 November 2012
Accepted 7 February 2013
Available online 24 February 2013

Keywords:
Nicotiana tabacum
Abiotic stress
B_6 vitamers
HPLC analysis

ABSTRACT

There are six different vitamin B_6 (VB_6) forms, pyridoxal (PL), pyridoxamine (PM), pyridoxine (PN), pyridoxal 5′-phosphate (PLP), pyridoxamine 5′-phosphate (PMP), and pyridoxine 5′-phosphate (PNP), of which PLP is the active form. Although transcriptional regulation of the genes involved in the *de novo* and salvage pathways of PLP syntheses after stress treatments has been described for *Arabidopsis thaliana* and tobacco plants, it remains open as to whether this in turn affects VB_6 levels. In this study, the effects of chilling, UV radiation, intensity of illumination, osmotic pressure, oxidative and drought stresses on the abundance of different B_6 vitamers in tobacco plants were examined by using high performance liquid chromatography (HPLC). The abiotic stressors resulted in significant increase of PLP, and caused some corresponding changes with PL and PN. The highest increase of PLP was 2.5-fold compared to the control plants, followed by a continuous decline back to the control levels. These changes are presumably caused by the regulation and control mechanism on the VB_6 metabolism in plants.

1. Introduction

Vitamin B_6 (VB_6) is a collective term for one group of pyridine compounds, including pyridoxal (PL), pyridoxamine (PM), pyridoxine (PN), and their phosphorylated derivatives: Pyridoxal 5′-phosphate (PLP), pyridoxamine 5′-phosphate (PMP), and pyridoxine 5′-phosphate (PNP). PLP is the active form of VB_6 and acts as an important coenzyme in over one hundred different cellular reactions and processes. VB_6 is synthesized *de novo* by two different enzymatic pathways [1]. The DXP (1′-deoxy-D-xylulose-5′-phosphate)-dependent pathway was extensively studied in *Escherichia coli* and for a long time assumed to be ubiquitous. In the DXP-independent pathway, PLP is directly synthesized by a complex of two proteins (PDX1 and PDX2). This pathway is widely distributed in nature, except for animals. In addition to the *de novo* pathways, a "salvage pathway" is found in all organisms, and functions to convert the six different vitamer forms between each other by a set of enzymes [2]. In plants, *PDX1* and *PDX2* have been isolated and characterized in tobacco [3], *Arabidopsis thaliana* [4,5], and *Ginkgo biloba* [6]. As for salvage pathway, genes encoding PL kinases have been cloned and characterized in both *A. thaliana* and wheat [7,8], and that of PNP/PMP oxidase has been cloned from *A. thaliana* [9,10]. A putative PL reductase has been identified in *A. thaliana* based on sequence homology with the protein in yeast [11], and a nonspecific acid phosphatase responsible for hydrolysis of all three phosphorylated B_6 vitamers has been purified and characterized in tobacco [12]. Furthermore, enzymatic conversion between PM and PL has also been found in tobacco [13].

The importance of VB_6 as a cofactor is well established, but only in the last 15 years have the additional functions of VB_6 as efficient antioxidants and factors able to increase resistance to biotic and abiotic stresses been demonstrated. VB_6 may play a crucial role in protecting cells from oxidative stress because VB_6 exhibits antioxidant activity that even exceeds that of vitamins C or E [14–17]. Loss of *A. thaliana* PDX1 causes hypersensitivity towards treatment with reactive oxygen species [18], and *A. thaliana pdx1* mutants are hypersensitive towards salt and UV-B treatments [19]. The *A. thaliana sos4* mutant with defect in PL kinase is highly sensitive to salt stress and cold treatment [20]. In addition, VB_6 deficient plants display increased sensitivity to high light and photo-oxidative stresses [21]. Moreover it is noteworthy that PDX1, PDX2 and PL kinase gene expressions have been found to be regulated in response to abiotic stressors including drought, chilling, UV-B treatment, ozone and abscisic acid treatment [3,5,20,22]. It raises the important question whether stress treatments in turn affect VB_6 levels in plants. However, the researches on this aspect are seldom discussed. Thus,

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http://dx.doi.org/10.1016/j.plaphy.2013.02.010

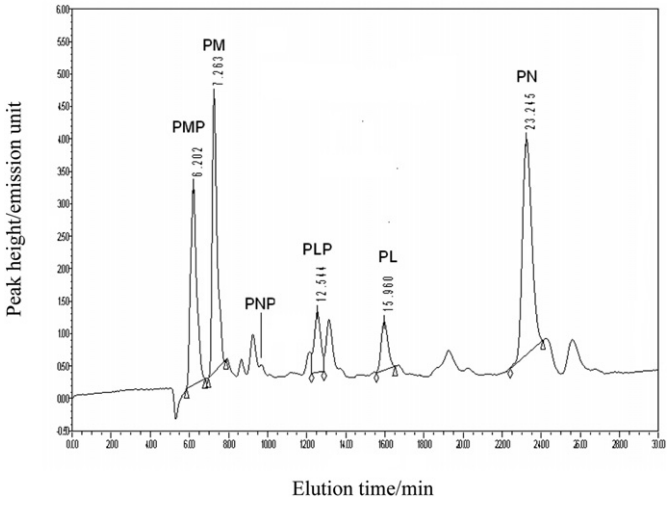

Fig. 1. HPLC pattern of leaf B_6 vitamers from tobacco plants grown on MS basal media.

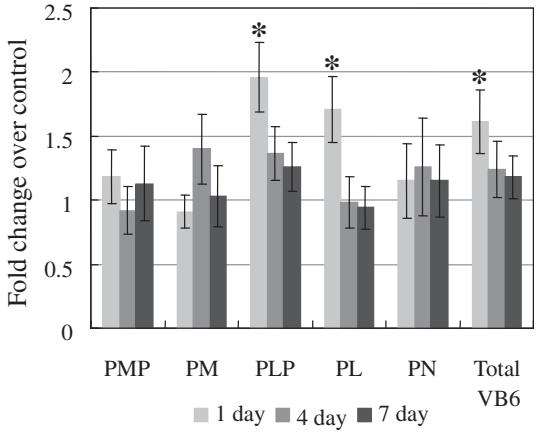

Fig. 2. Levels of leaf B_6 vitamers in tobacco plants after chilling stress. HPLC analysis showed the abundance of B_6 vitamers in *Nicotiana tabacum* after decreasing temperature from 25 °C to 5 °C for 1, 4, and 7 days. B_6 vitamer abundance is shown as the average fold change in chilling stressed plants (5 °C) over control plants (25 °C) from three experiments. Error bars represent standard deviation. Student's *t*-test was used to determine if there is statistical difference between experimental and control groups. Significantly different results are marked with asterisks ($P < 0.05$).

the aim of this study is to use responsive analyses to investigate how the changes of different B_6 vitamers take place in plants following different abiotic stresses. The effect of stress treatments on the abundance of different B_6 vitamers in tobacco plants was examined by using high performance liquid chromatography (HPLC). The results showed that content of certain B_6 vitamers changed under different stress conditions, and these changes in vitamer levels indicated that there is a regulation and control mechanism on the VB_6 metabolism in plants.

2. Results

2.1. HPLC analysis of B_6 vitamers in tobacco leaves

Pure standard B_6 vitamers were well-separated by HPLC. The retention times of PMP, PM, PNP, PLP, PL and PN were 6.2, 7.2, 9.5, 12.5, 15.9 and 23.2 min, respectively. PMP as well as PM emits relatively the strongest fluorescence, followed by PNP, PN and PL, with PLP having the weakest fluorescence under the same conditions. After treatment with KCN, the fluorescent signal of PLP was enhanced over 10-fold due to the formation of pyridoxic acid 5′-phosphate (PIC-P), and the retention time of PIC-P was 13.2 min (data not shown). Fig. 1 is the HPLC chromatogram of leaf B_6 vitamers in tobacco plants grown on Murashige and Skoog (MS) basal media, and the levels of B_6 vitamers in tobacco leaves are given in Table 1. The level of PN was higher in tobacco plants grown on the MS basal media, while that of PLP was higher in tobacco plants by soil culture.

2.2. Changes of B_6 vitamer levels in tobacco plants subjected to abiotic stress

2.2.1. Chilling stress

The effect of various abiotic stresses on the abundance of different B_6 vitamers in tobacco leaf tissue was analyzed using the HPLC method noted above. Plants were also monitored for phenotypic changes to abiotic stress. To test the effect of chilling stress on the abundance of the different B_6 vitamers, tobacco plants were grown in a 5 °C chamber. The abundance of different B_6 vitamers in leaf tissue was determined on days 1, 4, and 7 after stress treatment and compared to the control plants maintained at 25 °C. Results are shown in Fig. 2. An initial increase in vitamer level on day one of treatment was observed for PLP and PL followed by a continuous decline back to control levels on the fourth and seventh days. PLP showed the greatest increase compared to control levels with a 2-fold change. Tobacco plants subjected to chilling stress for 7 days showed normal growth without observable changes.

2.2.2. UV radiation treatment

For UV radiation stress, tobacco plants grown in a growth chamber under 2500 lux light with 14 h photoperiod were placed under the UV radiation of superclean bench, and B_6 vitamers in tobacco leaves were measured after 6, 12, and 24 h of UV radiation treatment. The levels of PLP, PN and total VB_6 showed a similar pattern reaching the highest fold change over controls early in the stress treatment (6 h), reaching a maximum 2.5-fold increase over control, followed by a return to control levels from 12 h continuously (Fig. 3). In addition, effects of 24-h light, 24-h dark, and weak light stresses (800 lux light with 14 h photoperiod for two weeks) were also analyzed. The PN level in tobacco plants kept in the dark for 24 h increased about 2-fold, and all other B_6 vitamer levels showed no significant change compared to control plants (data not shown). After 12 h of UV radiation treatment, the upper leaf of tobacco plants appeared brown, and the whole plants were brown in color by 24 h. When grown in a weak light chamber, the leaves began to turn yellow after a week, and showed symptom of excessive vegetative growth after two weeks.

Table 1
Vitamin B_6 contents in tobacco leaves determined by high performance liquid chromatography analysis (μg/g fresh weight).

Culture	PMP	PM	PLP[a]	PL	PN	PNP[b]	Total VB_6
Tissue	0.42 ± 0.09	0.54 ± 0.05	1.74 ± 0.13	0.40 ± 0.05	1.64 ± 0.31	Trace	4.75 ± 0.21
Soil	0.30 ± 0.07	0.23 ± 0.01	3.82 ± 0.39	0.38 ± 0.02	0.52 ± 0.13	Trace	5.26 ± 0.41

[a] The amounts of PLP were determined after KCN treatment.
[b] No significant elution peak could be used for quantitative analysis. The data are the average ± standard deviation of three biological replicates with three technical repeats.