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NAD KINASE ACTIVITY IN WHEAT LEAVES UNDER WATER DEFICIT

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Total NAD kinase activity remained unaltered in the drought non-adapted wheat leaves under water deficit, but gradually decreased with water deficit in the adapted ones. The share of the calmodulin-dependent enzyme was significantly higher in the drought-hardened than in non-hardened plants; however, under severe water deficit the activity of the enzyme dropped by half. It seems, therefore, that NAD kinase activity does not limit phosphorylation of NAD in dehydrated plant tissues.

Primary effects of water deficit in leaf tissues are caused mainly by the reduction of protoplast volume during cell dehydration [1]. The increased solute concentrations disturb plant metabolism by changing the substrate/product ratios and enzymatic activities. However, pre-dehydrated plants (drought-hardened) are able to maintain relatively intense metabolism under severe dehydration [2-5].

Our previous experiments [6] showed that the total pyridine nucleotide pool decreased gradually with the increasing water deficit in the leaves of non-hardened plants, but the molar concentration remained practically at the same level. The ratio of pyridine nucleotides to their phosphates increased from about 0.6 in the control non-hardened plants to about 2 at severe water deficit, the increase being mostly due to the increased level of NAD and decreased level of NADPH. In hardened plants, these changes were significantly less pronounced than in the non-hardened plants. These observations led to the supposition that the activity of NAD kinase (EC 2.7.1.23) responsible for conversion of pyridine nucleotides to their phosphorylated derivatives may be depressed by water deficit.

The aim of the present work was to examine the effect of water deficit on NAD kinase activity in the leaves of wheat adapted and non-adapted to cell dehydration.

MATERIAL AND METHODS

Biological material. Plants of spring wheat (*Triticum aestivum* L. ver. milturum cv. San Pastore) were grown in a growth chamber at 1.2×10^5 erg $cm^{-2}s^{-1}$ irradiance, 16 h photoperiod, and at high soil moisture. Control non-stressed plants were grown under these conditions throughout the whole experimental period. A part of the plants were drought-pretreated (drought--hardened) during the early stage of leaf development (i.e. during tissue differentiation). The plants (drought-pretreated and non-pretreated) were subjected to soil drought conditions leading to 20, 40 and 60% water saturation deficit of the fully matured, non-senescent fifth leaves. The water saturation deficit (WSD) was calculated according to Stocker [7]. Two series of independent experiments were carried out and three independent batches of leaf material were collected at each level of water deficit.

Assay of NAD kinase. The material was ground in liquid nitrogen and about 200 mg of tissue was extracted by the modified method of Allen & Trewavas [8] with buffer A (1:10, w/v) containing 1 M KCl, 1 mM EDTA, 50 mM Tris/HCl, pH 7.4, 2 mM $MgCl_2$ and 2.5% (w/v) PVP. The supernatant was passed through a Sephadex G-25 column equilibrated with buffer A, and was diluted with the same buffer. All procedures were performed at 4°C.

The NAD kinase activity was assayed by the modified method of Matsumoto *et al.* [9]. The incubation mixture contained 100 µl of 0.5 M Tricine/KOH (pH 8.0) 25 µl of 50 mM $CaCl_2$, 20 µl of 100 mM $MgCl_2$, 15 µl of 100 mM ATP, 30 µl of 50 mM NAD and 250 µl of the extract. The volume was adjusted to 600 µl with H_2O and the samples were incubated at 37°C for 30 min with shaking. At the end of the incubation the mixture was chilled in ice and 100 µl of 1 M HCl was added. The acidified samples were neutralized with 100 µl of 1 M NaOH and clarified by centrifugation.

The NADP level in the supernatant was determined enzymatically [10]. Absorbance at 570 nm was measured using Beckman spectrophotometer. Calmodulin-independent activity was assayed in the presence of 2 mM EDTA and absence of $MgCl_2$ and $CaCl_2$. The calmodulin-dependent activity was calculated by subtracting the calmodulin-independent activity from the total activity of the enzyme saturated with calcium [8].

RESULTS AND DISCUSSION

Two types of NAD kinase (EC 2.7.1.23) have been found in plants: calmodulin-dependent and non-dependent enzymes [11, 12]. The enzyme is localized mainly in chloroplasts (63% of the total content in wheat leaves), which contain two pools of NAD kinase: an envelope calmodulin-stimulated activity (10.8%) and a stromal (52.9) calmodulin-insensitive activity [13]. It has also been reported that NAD kinase is located in cytoplasm (35%) and

mitochondria (1%) of wheat leaves and that both forms are present in these compartments. The light-induced conversion of NAD to NADP in the chloroplast is catalysed exclusively by the photoactivated calmodulin--independent NAD kinase using ATP produced by photophosphorylation [11, 12, 13].

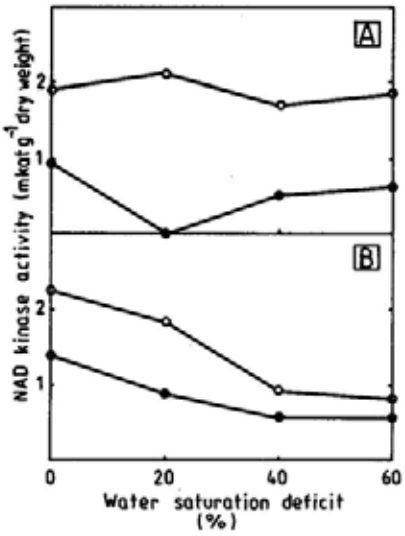

Fig. 1. The activity of NAD kinase in the non-hardened (A) and drought-hardened (B) wheat leaves under water deficit. Total NAD kinase activity (○); calmodulin-dependent activity (●)

As shown in Fig. 1, the total NAD kinase activity prior to subjecting the plants to water deficit was approximately the same in control leaves of non-hardened and hardened plants. However, in the control hardened leaves (Fig. 1B) the calmodulin-dependent NAD kinase activity was somewhat higher (65% of the total activity) than in the non-hardened leaves of control plants (45% of total activity). In both cases, the water deficit influenced the activity of the enzyme but in a different manner: unexpectedly, total NAD kinase activity remained practically at the same level in the non-hardened plants whereas it was reduced in the hardened ones. In the former plants, the calmodulin--dependent NAD kinase activity dropped to zero at mild water deficit but reappeared at 40 and 60% WSD and constituted about 1/3 of the total activity. In hardened plants the activity of the calmodulin-dependent enzyme decreased gradually with the increasing water deficit but at the stronger water deficit still constituted about 75% of the total activity. Participation of the two NAD kinase forms differed in hardened and non-hardened plants; e.g. the share of calmodulin-dependent NAD kinase was higher in the total activity in hardened plants but the absolute values were comparable at 40 and 60% WSD in both types of plants. At 20% WSD the calmodulin-dependent NAD kinase in non--hardened plants was undetectable and reappeared at the more severe water

deficit. This surprising and reproducible derepression of the calmodulin-dependent activity is consistent with the same phenomenon observed at the sublethal Al stress [14]. The mechanism of this depression is not known; it might be ascribed, as previously suggested [14], either to the formation of a new calmodulin-dependent isoform or depression of the enzyme repressor.

The calmodulin-independent NAD kinase activity decreased with increasing drought in hardened plants in spite of a higher NADP(H)/NAD(H) ratio and a higher level of ATP [6], whereas it remained practically unaltered in the non-hardened plants. Is this difference due in the hardened plants to a significantly lower concentration of NAD [6], the enzyme substrate? However, to our knowledge, the data on induction of NAD kinase by NAD are not available. No information on substrate affinities is available, either. It is not clear, either, whether the higher level and higher share of the calmodulin-dependent activity in the hardened plants than in non-hardened ones at stronger water deficit might be of any importance in drought tolerance. Participation of this activity in chloroplast is low but in cytosol it is equal to that of the calmodulin-independent form [13]. However, judging from the total NAD kinase activity in the hardened and non-hardened plants, the results obtained imply that the synthesis of NADP by NAD kinase does not limit the anabolic processes in leaf tissues under water deficit. It seems that the decreased amount of reducing equivalents is rather more restrictive for metabolism of the plant under water deficit [6].

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