THE INFLUENCE OF EXOGENOUS SPERMIDINE ON POLYSOMES AND RNase ACTIVITY IN WHEAT LEAVES UNDER WATER STRESS CONDITIONS

SHORT COMMUNICATION

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ABSTRACT

It was found that spermidine (5mM) delivered to wheat seedlings through their roots prior to water stress affected the state of polysomes and ribosomes under water stress conditions, which was manifested in stabilization of 80 S ribosomes and in reduction of polysome fraction. Besides, it was observed that pretreatment with spermidine decreases RNase activity in plants exposed to water stress.

KEY WORDS: polysomes, ribosomes, spermidine, RNase, wheat, water stress

INTRODUCTION

Water deficit in vegetative tissues decreases the level of polysomes through their disintegration. The effect is associated with a decrease in the capacity for protein synthesis under stress conditions (Mortilla et al. 1973, Rhodes and Matsuda 1976, Górecki and Grzesiuk 1978, Mason et al. 1988). According to Armstrong and Jones (1973) a decreased water content in vegetative tissues leads to reduction of ribosome 80 S content on rough reticulum.

Drought conditions and a noticeable water deficit in plants increase RNase activity, which is associated with a decrease in protein and nucleic acid synthesis (Maranville and Paulsen 1972).

Water stress induces a significant accumulation of polyamines in plant (Kubiś and Krzywański 1986, 1989, Turner and Stewart 1986). It is suggested that they may have fulfilled protective functions under water stress conditions through stabilization of nucleic acids and ribosomes (Smith 1985, Bagni 1986, Sińska 1986, Bagni and Torrigiani 1992). Earlier studies showed that exogenous spermidine in 5 mM concentrations may attenuate some negative effects caused by drought in wheat plants.

The main purpose of this paper was to investigate whether spermidine delivered through the roots to wheat seedlings may play a protective role in relation to the state of polysomes under water stress conditions.

MATERIAL AND METHODS

Plant material

Wheat (*Triticum vulgare* cv. Eta) seedlings at the 3rd-leaf stage of development were divided into two portions: the first group soaked in 1 mM phosphate buffer at pH 5.8 for 24

hours, and the second group soaked during the same time in the same buffer containing 5 mM spermidine. While soaking the plants were maintained in a controlled growth chamber (at 22°C, 65-70% humidity, and with continuous fluorescent light at 90 $\mu\text{mol} \times \text{m}^{-2} \times \text{s}^{-1}$ intensity). After 24 hours the seedlings of the first portion were again divided into two parts, the first of which was left in the buffer (control – not treated), and the second one was transferred into a dry beaker after a previous blotting of roots (stress – not treated). Plants of the second portion were also divided into two portions: the first of them after blotting the roots was transferred into the spermidine-containing buffer (control – treated with Spd), whereas the other, after blotting the roots, was put into a dry beaker (stress – Spd treated). The beakers with plants were placed into an air-conditioned chamber for 10 hours at 24°C and 65-70% humidity. After 10 hours the relative water content (RWC) of the 2nd expanded leaf in the control and stressed plants was determined by a modified method of Weatherley (1950) according to Slavik (1974). From the remaining control and stressed plants the second fully expanded leaf was excised and immediately frozen in liquid nitrogen and stored in dry ice. Polysomes in this material were analyzed and RNase activity was determined.

Isolation of polysomes

Polysomes were isolated according to the procedure of Davies et al. (1972), Davies and Larkins (1972), Gwóźdź and Deckert (1989) with some modifications. For extraction 1 g of frozen leaves were taken. They were ground in a mortar chilled to 2°C, with the addition of quartz sand and 20 ml extraction buffer containing 250 mM sucrose (RNase-free), 200 mM Tris-HCl at pH 8.5, 60 mM KCl, 30 mM MgCl₂ and 2% Triton X-100. The homogenate was centrifuged at 30 000 $\times$ g for 30 minutes. The supernatant was layered onto a 2 ml cushion of 1.5 M sucrose in the buffer containing: 40 mM

Tris-HCl, pH 8.5, 20 mM KCl, 10 mM MgCl₂ and centrifuged for 90 minutes at $200\,000 \times g_{\max}$. The precipitate was resuspended in 100 μ l 40 mM Tris-HCl at pH 8.5 containing 20 mM KCl and 10 mM MgCl₂. 50- μ g RNA was layered onto ca. 6 ml continuous sucrose gradient (10-20%) composed of 20 mM Tris-HCl, pH 8.5, 20 mM KCl and 10 mM MgCl₂, and then, centrifuged at $192\,000 \times g_{\max}$ in a Beckman SW 50 rotor for 60 minutes. The profiles of the ribosomal preparations were determined by measuring the A₂₅₄ with an UA-5 flow recorder (ISCO, Lincoln, NE, USA).

Polysome and ribosome percentages were calculated from the surface of sucrose gradient profiles of ribosome and polysome fractions. The percentages of ribosomal subunits (40 S and 60 S) and ribosomes 80 S were calculated from the ribosome region surface of sucrose profiles.

RNase activity

RNase activity was determined by the method of Tuve and Anfinsen (1960) in the extracts from the control and stressed leaves prepared as follows: 1 g of previously frozen leaves was ground in a mortar with the addition of quartz sand with 6 ml buffer 50 mM Tris-HCl at pH 7.5. The homogenate was centrifuged at $30\,000 \times g$ for 30 minutes. In the obtained extracts the level of RNase activity was determined. One unit of activity was the amount of enzyme which produces ΔE of 1.0 at 260 nm under experimental conditions. Its activity was expressed as unit $\times$ mg protein⁻¹ $\times$ 30 min⁻¹.

Protein content

Protein content in leaf extracts was determined by the method of Bradford (1976). The experiments were carried out in two series. Two isolations of polysomes for each experimental combination were made in each experiment. The activity of RNase was determined in 3 replications for each combination and the relative water content was estimated in 4 replications. Data are expressed as mean $\pm$ standart deviation.

RESULTS AND DISCUSSION

Fig.1 presents profiles of sucrose gradient ribosomes and polysomes from the leaves of wheat seedlings exposed to

water stress and treated with spermidine before the stress. A thorough analysis of sucrose profiles is presented in Table 1. Stressed plants were found to have a negligible decrease of polysomes as compared to the controls. This is indicative of plant resistance to the applied water stress. Possibly, it is related to the physiological state of the vegetative material, since the analyses were performed on fully expanded leaves, which are more resistant to drought (Bewley et al. 1983, Mason et al. 1988). A decrease in the polysome level was higher in plants treated with spermidine before the stress and then exposed to it. That was not caused by RNase increase under conditions of water deficit (Table 1), but could be the result of plant pretreatment with 5 mM spermidine.

Many papers on stimulation of *in vitro* protein biosynthesis by polyamines have appeared. Spermidine or spermine are added with other components to prepare a mixture giving optimal activity and fidelity of translation (Tabor and Tabor 1984). It is assumed that the applied concentration of spermidine was not suitable for polysome stabilization under experimental conditions. The concentration of spermidine used for treatment of wheat plants before the stress was chosen on the basis of earlier investigations (Kubiś et al.1991), which showed a decrease in the activity of proteases and a reduction of membrane permeability in plants exposed to drought and treated with 5 mM spermidine as compared to untreated plants.

As follows from Fig.1 and Table 1, the applied water stress caused a marked decrease in the number of ribosomes 80 S and an increase in ribosomal subunits in stressed plants in comparison to the controls. Calculations showed that the increase in the ribosome number was larger in plants exposed to drought, not previously treated with spermidine. It seems that plant pretreatment with 5 mM spermidine resulted in stabilization of ribosomes under conditions of water stress. It is well-known that polyamines bind to ribosomes and facilitate the association of ribosomal subunits (Tabor and Tabor 1984).

The results of RNase activity measurements are given in Table 1. Only a slight but significant increase in the studied enzyme activity was observed under stress conditions, which is associated with statistically significant reduction of water content in the leaves exposed to stress. A significant decrease in the water content of leaves in conjunction with obvious

TABLE 1. The analysis of profiles of sucrose gradient ribosomes and polysomes, activity of RNase (units $\cdot$ mg protein⁻¹ $\cdot$ 30 min⁻¹) and relative water content (RWC) in the leaves of wheat seedlings exposed to water stress and treated with spermidine (Spd) before stress. Data are expressed as mean $\pm$ standard deviation.

Plants	RWC %	RNase activity	% content of polysomes and ribosomes calculated from the surface of sucrose gradient profiles		% content of ribosomal subunits (40 S + 60 S) ribosomes 80 S calculated from the surface of ribosome region of sucrose profiles	
			ribosomes	polysomes	40 S + 60 S	80 S
not treated						
control	97,14 $\pm$ 0,85	7,60 $\pm$ 0,38	46,78 $\pm$ 2,72	53,20 $\pm$ 1,56	41,38 $\pm$ 1,02	58,28 $\pm$ 1,44
stress	83,60 $\pm$ 2,61	8,74 $\pm$ 0,38	50,29 $\pm$ 3,43	49,10 $\pm$ 3,38	81,04 $\pm$ 0,86	18,10 $\pm$ 0,97
treated – Spd						
control	97,50 $\pm$ 1,34	6,89 $\pm$ 0,70	45,34 $\pm$ 3,40	54,30 $\pm$ 3,49	44,52 $\pm$ 2,51	55,40 $\pm$ 2,74
stress	85,50 $\pm$ 1,58	6,84 $\pm$ 0,40	59,20 $\pm$ 2,52	40,64 $\pm$ 2,42	74,84 $\pm$ 1,43	26,44 $\pm$ 1,31

Fig. 1. Ribosome and polysome profiles of sucrose gradients from leaves of wheat seedlings exposed to water stress and treated with 5 mM spermidine before stress.

drought symptoms is the cause of a drastic increase in RNase activity (Maranville and Paulsen 1972, Blekhan 1978, Yi and Todd 1979). The activity of RNase in stressed plants, previously treated with spermidine, was on the level of that of the control plants treated with spermidine, but was statistically significantly lower than that of stressed plants not treated with polyamine. The obtained results are in agreement with literature reports on this subject (Merlo et al. 1988, Isola and Franzoni 1989) and constitute a supplement to earlier studies in this field (Kubiś et al. 1991). It should be mentioned that results obtained by Isola and Franzoni (1989), who found that exogenous spermidine strongly inhibits RNase activity in injured potato tuber slices, deserve attention. A decrease in this enzyme activity was associated with a reduction of RNase protein. It cannot be excluded that this fact may be related with a decrease in the function of polysomes under these conditions.

Summing up the results of our experiments, it should be inferred that exogenous spermidine in 5 mM concentration had a stabilizing effect on ribosomes 80 S and decrease the polysome level under water stress conditions. A continuation of studies in this field is undoubtedly necessary.

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WPŁYW EGZOGENNEJ SPERMIDYNY NA STAN POLISOMÓW I AKTYWNOŚĆ RNazy W LIŚCIACH PSZENICY W WARUNKACH STRESU WODNEGO

STRESZCZENIE

Stwierdzono, że spermidyna (5 mM) podana przedstresowo przez korzenie do siewek pszenicy wpłynęła na stan polisomów i rybosomów w warunkach stresu wodnego. Uwidocznili się to w stabilizacji rybosomów 80 S oraz obniżeniu frakcji polisomów. Ponadto zaobserwowano, że wstępne traktowanie spermidyną obniża aktywność RNazy u roślin poddanych stresowi wodnemu.

SŁOWA KLUCZOWE: polisomy, rybosomy, spermidyna, RNaza, pszenica, stres wodny.