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Acid phosphatase activity in maize (*Zea mays* L.) seedlings treated with sodium humate

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Abstract

Sodium humate increased the acid phosphatase activity of fraction II of imbibing maize caryopses and suppressed the enzyme activity in fraction I. Removal of the testa changed the stimulating action of humate on the acid phosphatase activity of fraction II into an inhibiting one and intensified its unfavourable effect on this enzyme's activity in fraction I. Sodium humate inhibited the acid phosphatase activity of this fraction from both leaves and roots. The results obtained are in agreement with the theory of free radicals of humus substances being involved in action on biomembranes.

Key words: Zea mays, acid phosphatase, sodium humate

INTRODUCTION

When studying the effect of sodium humate on phosphorus intake by young maize plants we found (Tichý 1984) that this humus substance can, with the endosperm removed, slow down the sorption of the phosphate ion from the medium. Since in such cases, however, the production of plant matter remained unaffected (Tichý 1985), it was necessary to assume that one of the ways in which humate acts on the plant is the stimulation of acid phosphatase activity. Namely, this enzyme represents an essential part of phosphorus reutilization pathways in plants, since it contributes to the formation of the inorganic phosphorus (P_i) pool in the cell and to the maintenance of its level. In this function, the action of acid phosphatase

is connected with the control mechanisms of the active transport of phosphate ions across the plasmalemma (Pedersen and Wehrle 1982). Insufficient phosphorus influx into the cell leads to a more intense activity of acid phosphatase as demonstrated by Besford (1980), Barrett-Lennard et al. (1982), Kummerová (1983), Kummerová and Buczek ((1983), Press and Lee (1983), Dracup et al. (1984), Lisiak (1984b) and McLachlan (1984). This involves not only changes of the overall activity of acid phosphatase but also changes in some fractions of the tissue homogenate.

It is well known that humus substances influence the transport function of the plasmalemma as well as the rate of metabolism of mineral nutrients (Lisiak 1984a) and reutilisation. One can therefore expect that their influence will also be reflected in changes of acid phosphatase activity, a phenomenon also observed in other enzymes. However, literary data concerning the effects of humus substances on phosphatase activity show disunity and contradiction (Prát 1964). Therefore, a further investigation of the relationships between humus substances and acid phosphatase activity under the conditions of phosphorus deficiency is of importance (Lisiak 1984b) not only for a theoretical clarification of the interaction of soil organic matter and plant metabolism but also for the possibilities of practical uses of measuring the activity of this enzyme for the purpose of timely diagnosing phosphorus deficiency in cultured plants (Besford 1979).

In this paper we present the first part of the results we obtained in this field of study. It is devoted to the acid phosphatase activity in maize caryopses and seedlings in a phosphate-free medium containing sodium humate.

MATERIAL AND METHODS

Two experiments were carried out. In the first, maize caryopses (*Zea mays* L., cv. CE 170) were imbibed both in distilled water and in humate solutions with concentrations 50 and 100 $mg \times dm^{-3}$. Prior to imbibition, one-half of the number of caryopses in each variant were deprived mechanically, using a scalpel, of their testae. After 24-hr imbibition, the activity of acid phosphatase was determined in caryopses in all variants.

In the second experiment, caryopses of the same maize cultivar with their testa retained, imbibed for 24 hours and germinated for 72 hours on filter paper in distilled water as well as in a solution of sodium humate at a concentration of 50 $mg \times dm^{-3}$. Maize seedlings, after germination on filter paper, were placed for two days on cotton gauze stretched over glass crystallizing dishes filled with distilled water and/or sodium humate solution. Then endosperm was removed and the germinated plants were cultured for 5 days in a solution of 2.5 mM $CaSO_4$, 1 mM $MgSO_4$

diluted 1:10 as well as in this solution with sodium humate added. Cultivation was carried out at a temperature of 25°C and at 16-hr daylight. Acid phosphatase activity was determined in the caryopses after 24 hours of imbibition, and in the roots and leaves of 4, 6 and 11 day old seedlings. The sodium humate preparation was obtained from peat by extracting with 0.1 M NaOH, precipitating the extract with 1 M HCl, washing the precipitate with water and removing hymatomelanic acid with the aid of hot ethanol. Dissolved in NaOH, humic acid was transformed into sodium humate which was used for the experiments. The preparation of humic acid so obtained contained 3.9% ash and the colour quotient Q 4/6 of its sodium salt was 8.3 (Tichý 1981).

Determination of acid phosphatase activity. 1 g fresh plant tissue was homogenized in a porcelain mortar in a 0.1 M solution of sodium acetate (pH 4.5) and 0.25 M sucrose. For 1 g of plant tissue, 10 cm^3 extraction solution were used. The homogenate was centrifuged at 1500 × g for 15 minutes. The sediment was discarded and the supernatant was recentrifuged at 18000 × g for another 15 minutes. After centrifugation, the sediment was suspended in 10 cm^3 of extraction solution (fraction I), and the supernatant was used afterwards, designated as fraction II (see also Kummerová 1983). Extraction and separation of the fraction took place at the temperature of 0-4 C. Preliminary experiments have shown that a further purification of the sediment (fraction I) by repeated centrifugation had no significant effect on its phosphatase activity. The incubation medium was composed of 2 cm^3 0.1 M sodium acetate pH 4.5 and 25 mM potassium chloride; 0.5 cm^3 3 mM p-NPP (p-nitrophenylphosphate) and 0.5 cm^3 enzymatic preparation of the given fraction. Incubation was done at 37°C for 30 minutes and after which the reaction was stopped by adding 1 cm^3 cool 20% TCA. The inorganic phosphate released from p-NPP was determined colometrically according to Fiske and Subbarow (1925). The activity of acid phosphatase was expressed in μmoles of released P_i per 1 g fresh weight per hour. This colorimetric determination was repeated 3 times for each sample of the same experiment. The numerical data given in the tables are the mean values of three separately conducted experiments (n = 3). They were treated statistically by the t-test (Weber 1972).

RESULTS AND DISCUSSION

The first part of the results comprises data on acid phosphatase activity in maize caryopses after a 24-hr period of imbibition in distilled water and solutions of sodium humate. One-half of the number of the caryopses examined in this experiment had been deprived of their testae. The results

obtained are presented in Table 1. The second part contains data on acid phosphatase activity in the leaves and roots of maize seedlings during the first 11 days of growth. The results obtained are summarized in Tables 2 and 3.

Table 1

Acid phosphatase activity in maize caryopses with testae and deprived of testae after 24 hours of imbibition in water and in sodium humate solution (NaHU). Statistical significance of differences between the variants with NaHU solutions and water is marked * for $P \leq 0.05$ and ** for $P \leq 0.01$

NaHU concentration, $mg \times dm^{-3}$	Acid phosphatase activity, $\mu mol\ P_i \times g^{-1}$ fresh weight $\times h^{-1}$			
	fraction I of caryopses		fraction II of caryopses	
	with testae	without testae	with testae	without testae
0 (water)	2.49	4.23	159.3	198.5
50	2.19	1.80**	188.8**	130.4**
100	1.93*	1.14**	180.6*	165.7**

Removal of the testa in caryopses imbibing pure water enhanced the activity of acid phosphatase of both fraction I and fraction II. In contrast, removal of the testa in caryopses imbibing sodium humate solution invariably produced a reduction of this enzyme activity. Changes in the phosphatase activities of the two subcellular fractions caused by removal of the testa were not parallel; this is manifested in the values of the ratio between the phosphatase activities of fraction II and fraction I (Table 3). Through the action of humate this ratio increased, particularly in the variants involving testa-free caryopses, as can be seen from the values given in Table 1.

In comparison with the acid phosphatase activity in the caryopses, the activity of acid phosphatase in the leaves and roots was generally higher on the 4th day of the experiment, but during the following days its pattern varied from variant to variant (Table 2). The acid phosphatase activity of fraction II declined dramatically in both leaves and roots without any stronger effect of sodium humate being observed. However, this decrease was not identical in leaves and roots as can be seen from Table 3, the ratio of the acid phosphatase activity in the leaves to that in the roots was lower in the humate-containing variant. The acid phosphatase activity of fraction I of the roots did not change in the variant without sodium humate from the 4th day of the experiment on, whereas it decreased dramatically in the variant with humate up to the 11th day of testing. The acid phosphatase activity of fraction I of the leaves rose in the presence of humate slowly, but evenly up to the 11th day. It rose sharply in the

Tabela 2

Acid phosphatase activity of fraction I and fraction II of caryopses, leaves and roots of maize seedlings during the first 11 days of cultivation in media with and without humate (50 mg NaHU $\times dm^{-3}$). Statistical significance of differences between the variants with NaHU solution and water is marked * for $P \leqslant 0.05$ and ** for $P \leqslant 0.01$

Treatment	Age of plants	Acid phosphatase activity, μmol $P_i \times g^{-1}$ fresh weight $\times h^{-1}$ of the fractions			
		I	II	I	II
Water Ca , Mg		caryopses			
	1 day	2.96	180.0		
		leaves		roots	
	4 days	3.87	363.7	7.09	304.2
	6 days	7.02	228.9	6.53	62.8
	11 days	7.15	88.0	6.55	15.3
NaHU Ca Mg		caryopses			
	1 day	2.76	203.0		
		leaves		roots	
	4 days	3.34	337.6	6.84	267.9
	6 days	3.92*	223.8	5.87	101.9**
	11 days	4.68**	90.2	3.74	14.8

humate-free variant to the 6th day to remain later at the attained level.

The computed characteristics (Table 3) show that the ratio between fraction II activity and fraction I activity increased generally in the leaves because of the action of humate. The ratio of activities of both fractions in the leaves to root activities was rather decreased in the presence of humate.

It can thus be concluded that the action of sodium humate on the activity of acid phosphatase observed in our experiments appears as generally inhibiting. This is in good agreement with the results of a recent paper by Lisiak (1984b) about the effect of sodium humate on the activity of acid phosphatase in tomato seedling leaves. At the same time, it does not matter whether the phosphorus deficiency in the medium is partial (Lisiak 1984b) or complete, which was the condition intended in this study.

In our experiments, the only exception is the stimulation of acid phosphatase activity of the cytosol fraction during the first day of the imbibition of the caryopses. Removal of the testa, which can be regarded as facilitating the penetration of humate solution into the endosperm and embryo, changed this effect even in this case into an inhibiting one.

Table 3

The ratio of acid phosphatase activity of fraction II to the acid phosphatase activity of fraction I in leaves and roots and the ratio of phosphatase activities of these fractions in leaves to activities of the same fractions in roots of maize during the first 11 days of cultivation with and without sodium humate (50 mg NaHU × dm $^{-3}$). Statistical significance of differences between the variants with NaHU solution and water is marked * for $P < 0.05$ and ** for $P < 0.01$

Treatment	Age of plants	The ratio of acid phosphatase activity in			
		the fractions		leaves:roots	
		II:I		of fractions	
Water Ca, Mg		caryopses			
	1 day	63.3			
		leaves	roots	I	II
	4 days	98.4	44.3	0.54	1.21
	6 days	33.6	9.8	1.10	3.71
	11 days	12.3	2.6	1.13	6.83
NaHU Ca, Mg		caryopses			
	1 day	75.9			
		leaves	roots	I	II
	4 days	105.6	40.8	0.52	1.30
	6 days	58.9	18.3	0.69	2.23*
	11 days	19.2**	4.0	1.26	6.45

However, humate-induced changes in acid phosphatase activity cannot be considered as an indirect consequence of a restricted or facilitated water intake by the caryopses (Prát 1964) at the beginning of germination, because a supplementary experiment did not exhibit any significant differences in the imbibition of maize caryopses in water and sodium humate solutions (50 and 100 mg × dm $^{-3}$) during the first 24 hours of germination.

A contribution to an interpretation of the phenomena observed is suggested by the fact that — leaving aside the absolute values — it is rather the activity of fraction I that is affected by humate action. The low absolute values of the activity of this fraction obviously afford the possibility of a more refined distinction of its relative changes. Our assumption, based on literary data (e.g. Karlson 1977, Metzler 1977), that this fraction mostly consists of mitochondria or fragments of membranes of mitochondrial origin testifies to the fact that humate interacts with functions following from the activity of membranes of this compartment rather than from the activity of cytosol systems. This corroborates the idea already proposed earlier (Tichý 1984), namely, that the effect of humate on living systems rests primarily on the alteration of physical and/or electrochemical properties

of biomembranes. In this case, it could be possible to attach key importance to the interaction of membranes with unpaired spins of humate macromolecules (Prát 1955, Flaig 1975).

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Wpływ humianu sodowego na aktywność kwaśnej fosfatazy w siewkach kukurydzy

Streszczenie

Humian sodowy zwiększał aktywność kwaśnej fosfatazy frakcji II sporządzonej z napęczniałych nasion kukurydzy oraz zmniejszał aktywność tego enzymu w preparatach frakcji I. Usunięcie okrywy nasiennej zmieniło dodatni wpływ humianu na aktywność kwaśnej fosfatazy frakcji II na ujemny i pogłębiło hamujący wpływ humianu na aktywność enzymu frakcji I. Humian sodowy hamował aktywność kwaśnej fosfatazy badanych frakcji, zarówno w pędach jak i korzeniach siewek kukurydzy. Otrzymane wyniki popierają teorię działania wolnych rodników substancji humusowej na błony plazmatyczne.