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EFFECT OF AMINES AND GUANIDINES ON ATPase FROM MAIZE SCUTELLUM

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Key Word Index—*Zea mays*; Gramineae; maize scutellum; ATPase; polyamines; guanidino-acetic acid; dodine; guazatine; sulfhydryl reagents; activation; membranes.

Abstract—The membrane-bound ATPase activity of excised maize scutellum is activated by polyamines, guanidino-acetic acid, guanidino-butyric acid, dodine, guazatine and sulfhydryl reagents as a result of their binding to the membrane. Arginine, agmatine, creatine and creatinine have no effect by themselves but decrease the effect of polyamines. ATPase present in the soluble fraction or the membrane-bound enzyme solubilized by Triton and deoxycholate is not affected by polyamines or guanidines. The activation by dodine and guanidino-acetic acid is potentiated by sulfhydryl reagents.

INTRODUCTION

The functions of polyamines and guanidines in controlling several plant processes including membrane-mediated responses have been reviewed in a previous paper [1]. In that paper we reported the control of membrane-bound peroxidase in maize scutellum by polyamines and guanidines. ATPases are also known to be localized in plasmalemma [2–6], mitochondria [7, 8] and chloroplasts [7] of plant cells. Membrane-bound ATPases are controlled by cations [9–11], polar lipids [12] and polyamines [8, 13–15]. Among the guanidino compounds, octylguanidine has been reported to inhibit ATPase activity [16]. ATPases from rabbit brain microsomes are not inhibited by any of the guanidino compounds except methyl guanidine which inhibits the Na^+K^+ ATPase [17]. In the present paper we report the effect of polyamines, guanidines and sulfhydryl reagents on membrane-bound ATPase in maize scutellum which has a plasmalemma-bound enzyme [5, 6].

RESULTS AND DISCUSSION

ATPase activity in maize scutellum when assayed on the day of excision (0 day incubation) (Table 1) increased with germination up to 9 days. Incubation of the excised scutella for different days increased ATPase activity continuously until it reached a maximum level (*ca* 27 nkat/g tissue). Longer periods of incubation of the scutella excised from 8–10 day germinated seedlings slightly decreased the ATPase activity. ATPase activity in the slices from 4-day germinated seedlings (8 nkat/g tissue) was *ca* 6% of the total activity present in the homogenate (131 nkat/g tissue). Wheeler *et al.* [5] have provided evidence that the enzyme assayed in maize scutellum slices is plasmalemma-bound ATPase only.

Incubation of slices from 4-day germinated seedlings with amines and guanidines showed (Table 2) that among the diamines, only putrescine and cadaverine activated ATPase by 42 and 28%, respectively. Out of the guanidino compounds tested, guanidino-acetic acid (GAA), guanidino-butyric acid, dodine and guazatine activated the enzyme by 40–60%, but arginine, agmatine, creatine and creatinine had no significant effect. None of these compounds, however, had any effect in the slices from 8-day germinated seedlings, which may be due to changes in membrane properties. The polyamine effect on ATPase activity was opposite to that obtained for membrane-bound peroxidase in the same tissue [1]. Polyamines have been reported to inhibit ATPases from human erythrocytes and pig kidney [13] and it was

Table 1. Development of ATPase activity in excised maize scutellum

Scutellum excised from seeds germinated for (days)	ATPase activity (nkat/g tissue) in excised scutellum incubated for (days)					
	0	1	2	3	4	5
1	2	7	8	9	13	14
2	4	9	10	12	16	16
3	4	11	13	16	17	20
4	8	14	14	18	20	22
5	9	16	18	23	23	24
6	11	17	19	23	24	26
7	15	18	20	24	28	26
8	16	21	23	27	26	23
9	18	24	25	28	25	23
10	19	26	28	26	24	23

Scutella were excised from seedlings germinated for different days starting from the day of transfer to Petri dishes and incubated up to 5 days. ATPase activity was assayed in the slices prepared from the excised scutella.

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Table 2. Effect of amines and guanidines on ATPase activity in maize scutellum slices

Compound	ATPase activity (% of control) in scutellum slices from seedlings germinated for (days)	
	4	8
1,2-Diaminoethane 2 HCl	92	99
1,3-Diaminopropane 2 HCl	97	100
1,4-Diaminobutane 2 HCl (putrescine)	142	100
1,5-Diaminopentane 2 HCl (cadaverine)	128	100
1,6-Diaminohexane 2 HCl	111	100
1,8-Diaminooctane 2 HCl	97	99
1,10-Diaminodecane 2 HCl	104	100
1,8-Diamino-4-azaoctane 3 HCl (spermidine)	148	104
1,12-Diamino-4,9-diazadodecane 4 HCl (spermine)	143	108
2-Amino-5-guanidine-*n*-valeric acid HCl (arginine)	115	100
1-Guanidino-4-aminobutane H_2SO_4 (agmatine)	114	100
N-Guanyl *N*-methyl glycine (creatine)	108	100
2-Imino-*N*-methyl hydantoin (creatinine)	100	96
N-Amidino-glycine (guanidino-acetic acid)	138	100
4-Guanidino-ethyl acetic acid (guanidino-butyric acid)	140	102
Guanidino-*n*-dodecane HOAc (dodine)	158	108
1,17-Diguanidino-9-azaheptadecane 3HOAc guazatine)	155	107

Scutellum slices from 4- or 8-day germinated maize seedlings were incubated with the compounds (2 mM) for 1 hr. After incubation the slices were washed with water and used for enzyme assay. Control activity on the fourth and eighth days was 7.8 and 15 nkat/g tissue, respectively.

suggested that the inhibition was not due to the displacement of Mg^{2+} for the Mg^{2+} ATPase. Inhibition of ATPase by polyamines has also been reported for Na^+K^+ ATPase in developing chick brain [14]. The inhibition was more pronounced with spermidine than with spermine and it was suggested that spermidine acts as an allosteric inhibitor. Contrary to these observations, Tashima *et al.* [15] and Peter *et al.* [8] have reported that spermine activates Na^+K^+ ATPase and the activation is prevented by excess Na^+ or K^+.

Polyamines and guanidines when tested at different concentrations showed activation up to 2 mM (Table 3) although it was more with slices compared to homogenates. Raising the concentration beyond 2 mM decreased the activation continuously in slices as well as in homogenates until it reached the control level at 5 mM, except with dodine where some activation was still evident in slices. A similar biphasic response of polyamines has been reported for ATPase activity by Peter *et al.* [13] but the displacement of Mg^{2+} from Mg^{2+}-ATP was excluded as a mode of action. In the present study also the reversal of the activating effect at high concentration of polyamines was not affected by increasing the Mg^{2+} concentration, showing that it was not due to displacement of Mg^{2+}.

Studies carried out to investigate the interaction between polyamines and guanidines showed that incubation of slices in water followed by polyamines and guanidines (Table 4) resulted in the activation of the enzyme by all the polyamines, GAA and dodine. However, incubation with polyamines first and then with GAA and dodine or vice versa did not show any additive effect. Arginine, agmatine, creatine and creatinine, which had no effect by themselves, considerably reduced the effect of polyamines whether the slices were incubated with these compounds first and then with polyamines or vice versa. Creatinine, which has no free guanidino group, could not abolish the effect of polyamines. Even though both polyamines and some guanidines activated ATPase, their effect was not additive, indicating that either they may bind to the membrane at the same site giving activation, which could not be enhanced by subsequent binding of the other compound, or if they bind at different sites, the maximum effect produced by the binding of the two is the same as that given by them individually.

Since membrane-bound ATPase was activated by polyamines and guanidines, it was of interest to study their effect on the subcellular fractions. The results of Table 5 show that the maximum activating effect of these compounds was for 10000 *g* and 40000 *g* fractions. The soluble enzyme which constituted *ca* 40% of the total activity in the homogenate was not affected by any of the compounds. The two particulate fractions were then treated with calcium chloride, Triton X-100 and sodium deoxycholate in an attempt to solubilize the enzyme. Contrary to the solubilization of peroxidase by Ca^{2+} [1], the ATPase was not solubilized. Triton and deoxycholate could solubilize (*ca* 40 and 60%, respectively) the ATPase activity from both fractions (Table 6). The residual enzyme was still activated by spermine and dodine but the enzyme solubilized by Triton or deoxycholate was not affected by either spermine or dodine. These results further prove that only the membrane-bound ATPase is affected by polyamines and guanidines and the effect is mediated through their binding to membranes. Incubation of slices with sulfhydryl reagents, cysteine, spermine and dodine showed (Table 7) that sulfhydryl reagents also activated ATPase and it was not affected by the subsequent treatment with spermine or vice versa. However, incubation of slices with sulfhydryl reagents followed by dodine or vice versa showed an additive effect. The results thus suggest that sulfhydryl reagents and polyamines may be binding at the same site and giving activation since it is not altered by treatment with one followed by the other. The sulfhydryl reagents and dodine may, however, bind at different sites to give an additive response. The control of ATPase activity by these compounds thus appears to be essentially similar to that of peroxidase described earlier [1] except that in the case of peroxidase polyamines cause inhibition rather than activation.

EXPERIMENTAL

Plant material. Maize seeds (*Zea mays* L. var. Ganga-2) were obtained from the National Seed Corporation, Baroda. Their germination, preparation of slices, homogenate and subcellular

Table 3. Effect of concentration of polyamines and guanidines on ATPase activity in maize scutellum slices and homogenate

Concentration (mM)	ATPase activity (% of control) with				
	Putrescine	Spermidine	Spermine	GAA*	Dodine
			Slices		
0.5	110	115	114	113	137
1	117	123	123	128	172
2	135	148	143	138	158
3	131	118	118	117	153
4	107	106	107	112	152
5	98	102	103	109	133
			Homogenate		
0.5	112	117	117	112	120
1	117	122	127	118	126
2	121	132	137	120	136
3	110	122	130	117	119
4	102	101	120	112	107
5	99	100	117	100	104

Scutella from 4-day germinated seedlings were used. Slices and homogenates were preincubated at 37° with the compounds at different concentrations for 1 hr. Control activity in slices and homogenate was 7.8 and 131 nkat/g tissue, respectively.

*GAA, Guanidino-acetic acid.

Table 4. Interaction between polyamines and guanidines for ATPase activity in scutellum slices

Treatment* 1st / 2nd	ATPase activity (%)									
	Water	Putrescine	Spermidine	Spermine	Arginine	Agmatine	Creatine	Creatinine	GAA†	Dodine
Water	100									
Putrescine	131				118	109	108	136	139	161
Spermidine	145				114	111	106	150	136	161
Spermine	152				112	109	106	155	142	166
Arginine	109	118	120	123						
Agmatine	117	114	117	119						
Creatine	108	122	108	123						
Creatinine	97	131	145	159						
GAA	138	136	149	145						
Dodine	166	158	159	148						

*Scutellum slices from 4-day germinated seedlings were preincubated (first treatment) for 1 hr in polyamines and guanidines (2 mM) at 37°. After washing in water, the polyamine-treated slices were incubated in guanidines and guanidine-treated slices were incubated in polyamines (second treatment) for 1 hr. After the second incubation the slices were washed in water and used for enzyme assay. A control group incubated in water for the same period was incubated and its activity (7.8 nkat/g tissue) was taken as 100%.

†GAA, Guanidino-acetic acid.

fractions and their treatment with various compounds were the same as described earlier [1].

Chemicals. ATP was purchased from Sigma. Other chemicals were obtained as described earlier [1].

Enzyme assay. ATPase activity was assayed essentially according to the method of ref. [5] by estimating the liberated phosphorus. The assay system consisted of 0.5 ml 0.1 M NaOAc buffer, pH 5.5; 10 μmol $MgSO_4$; 10μmol ATP and enzyme (0.1 ml 5% homogenate or 200 mg slices) in a total vol. of 3 ml. After incubation at 37° for 1 hr the reaction was terminated by 0.5 ml 10% TCA, and phosphorus was estimated in the centrifuged supernatant.

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Table 5. Effect of polyamines and guanidines on ATPase activity in subcellular fractions of maize scutellum

Compound	ATPase activity (% of control) in fractions 1000 *g*	10000 *g*	40000 *g*	105000 *g*	Soluble
Putrescine	107	133	135	109	100
Spermidine	110	147	141	124	100
Spermine	122	160	153	130	100
Arginine	103	107	106	93	103
Agmatine	100	107	106	94	100
Creatine	100	100	100	97	100
Creatinine	100	100	106	99	100
GAA*	100	127	135	109	100
Dodine	103	167	153	129	103

Subcellular fractions from scutella of 4-day germinated seedlings were prepared. These fractions had 7, 9, 22, 10 and 43% activity of the homogenate (131 nkat/g tissue). The fractions were incubated with polyamines and guanidines (1 mM) for 1 hr at 37° before the addition of other assay components for the enzyme activity test.

*GAA, Guanidino-acetic acid.

Table 6. Effect of spermine and dodine on ATPase activity in the 10000 *g* and 40000 *g* fractions treated with calcium chloride, Triton X-100 and sodium deoxycholate

Fraction	Treatment	ATPase activity (nkat/g tissue) in Residue Control	+ Spermine	+ Dodine	Supernatant Control	+ Spermine	+ Dodine
10000 *g*	—	23	30	30	0.3	0.3	0.3
	$CaCl_2$	23	30	30	0.5	0.5	0.5
	Triton X-100	14	18	17	10	10	10
	Deoxycholate	9	12	11	14	14	14
40000 *g*	—	28	37	36	0.2	0.2	0.2
	$CaCl_2$	28	36	36	0.3	0.3	0.3
	Triton X-100	17	22	21	11	11	11
	Deoxycholate	11	15	14	17	17	17

The 10000 *g* and 40000 *g* fractions from scutella of 4-day germinated seedlings were treated with calcium chloride (0.8 M), Triton X-100 (1%) and sodium deoxycholate (1%) in the grinding medium for 1 hr at 4° and then centrifuged at 10000 *g* and 40000 *g*, respectively, to separate the supernatant and residue fractions. These fractions were then incubated for 1 hr at 37° with spermine or dodine (1 mM) before the addition of other assay components for the enzyme activity test.

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Table 7. Interaction between iodoacetate, *p*-CMB, cysteine, spermine and dodine for ATPase activity in maize scutellum slices

Treatment 1st / 2nd	ATPase activity (%)					
	Water	Iodoacetate	*p*-CMB*	Cysteine	Spermine	Dodine
Water	100					
Iodoacetate	135				144	195
p-CMB	147				142	193
Cysteine	142	137	147		144	143
Spermine	142	142	142	142		
Dodine	142	200	200	140		

Scutellum slices from 4-day germinated seedlings were preincubated (first treatment) for 1 hr in iodoacetate (0.5 mM), *p*-CMB (0.5 mM), cysteine (0.5 mM), spermine (2 mM) and dodine (2 mM) at 37°. After washing in water, the iodoacetate- and *p*-CMB-treated slices were incubated in cysteine, spermine and dodine, while the cysteine-treated slices were incubated in spermine and dodine. Similarly, the spermine- and dodine-treated slices were incubated with iodoacetate, *p*-CMB and cysteine (second treatment) for 1 hr. After the second incubation the slices were washed with water and used for enzyme assay. A control group incubated in water for the same period was included and its activity (7.4 nkat/g tissue) was taken as 100%.

*p-CMB, *p*-chloromercuri-benzoate.

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