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EFFECT OF POLYAMINES ON TISSUE PERMEABILITY

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Key Word Index—*Beta vulgaris*; Chenopodiaceae; beet root; polyamines; spermidine; spermine; phytate; alcohol; betacyanin; membrane permeability.

Abstract—Spermidine and spermine decrease betacyanin efflux from the discs of beet root storage tissue probably by stabilizing the cell membrane. The efflux reversal by spermine is not achieved at 47° probably due to irreversible membrane damage. Phytate alone has no effect on the efflux but reverses the effect of spermine. The destabilization produced by alcohol is reversed by spermine at low concentration but at high concentration both act synergistically to enhance the efflux.

INTRODUCTION

Polyamines have been shown to protect bacteria against loss of viability [1, 2] by penetrating the cell wall and binding tightly to cytoplasmic membrane [3]. It is also suggested that the bacterial protoplast stabilization by polyamines is related to the cation-exchange property of the membrane [4]. Bernheim [5] found that polyamines preserved the integrity of *Pseudomonas aeruginosa* exposed to heat or streptomycin by decreasing the leakage of intracellular components. In mammalian cell lines, putressine and related amines serve as growth factors as a result of the stabilization of cellular membranes [6]. No data, however, are available for the effect of polyamines on plant membranes.

Betacyanin efflux from beet root cells has been widely used to demonstrate changes in membrane permeability in plants [7–11]. Many substances like alcohols and polycations [8–10], ammonium salts [12] and HCl [11] increase the efflux which can be reversed by Ca^{2+} ions. The role of Ca^{2+} ions in protecting membranes against the damaging effect of heat is also well documented [13]. In the present paper the effect of polyamines and related compounds on betacyanin efflux has been studied to explore the possibility that the observed effects might be related to their properties as stabilization–destabilization agents.

RESULTS AND DISCUSSION

Effect of temperature

Betacyanin efflux was increased with temperature at various time intervals (Fig. 1). The efflux which was low at 27° was not increased very markedly after 30 min but at 37° the efflux was significantly increased with period of incubation. The pigment leakage at 37° was inhibited by transferring the discs to 27°, showing that the temperature induced changes in the membrane at 37° are reversible. Similar observations have been reported by Toprover and Glinka [13]. At 47° a very brief period was sufficient to cause a sudden increased rate of pigment release, which is practically irreversible.

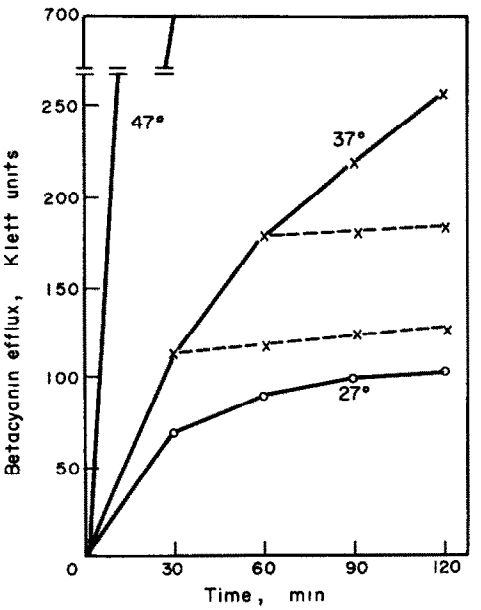

Fig. 1. Time course of betacyanin efflux at various temperatures. Broken lines show the effect when discs were transferred from 37° to 27°.

Effect of amines

The effect of various di- and polyamines on pigment leakage showed that spermidine and spermine are very effective in decreasing the efflux (Table 1). Of the diamines, putrescine was more efficient than agmatine and cadaverine. In all these cases, no further inhibition was observed by raising the concentration by 5-fold. The results suggest that polyamines have a powerful stabilizing effect on the beet root membrane, possibly as a result of their binding to the phospholipid components of the membrane. The efflux was decreased to *ca* 40% at 1 mM. The stabilizing effect of amines was not an unspecific one due to their cationic nature since several other monoamines, even at higher concentrations, did not

Table 1. Effect of amines on betacyanin efflux at 37°

Amines	Concentration (mM)	Betacyanin efflux (Klett units) at (min) 30	60	90	120
		126	200	260	280
Agmatine	1	125	188	220	230
	2	113	140	165	175
Putrescine	1	120	167	202	240
	2	88	107	122	130
Cadaverine	1	128	192	248	268
	2	118	150	185	195
Spermidine	0·2	130	210	270	284
	0·6	112	136	147	150
	1·0	108	127	132	138
Spermine	0·2	78	92	95	107
	0.6	66	87	92	105
	1.0	54	75	75	98

show any inhibitory effect. Siegel and Daly [8] have reported that polycations such as polylysine of high MW increase the efflux of betacyanin by destabilizing the membranes. Further kinetic studies by Siegel [11] showed that the increased betacyanin efflux by HCl, NH_4OH and polycations and a common mode of Ca^{2+}-ion antagonism was caused by polycations delivering a localized high concentration of substituted NH_3 (i.e. $(R\text{-}NH_2.H)^+$) to the cell surface. In our studies, the polyamines, though cationic in nature, do not seem to follow the above mechanism and they decrease the efflux by stabilizing the membrane. Ca^{2+} ions follow a pattern similar to that of polyamines though at a higher concentration.

The stabilization of betacyanin leakage by spermine was not significantly affected by temperature when studied at 27° and 37° (Fig. 2). However, it was completely abolished at 47° within 30 min. This may result from irreversible damage of the cell membrane at higher temperatures.

Effect of phytate

Results reported above suggested that polyamines must be binding with the anionic components on the membrane. Polyamines are also known to bind with

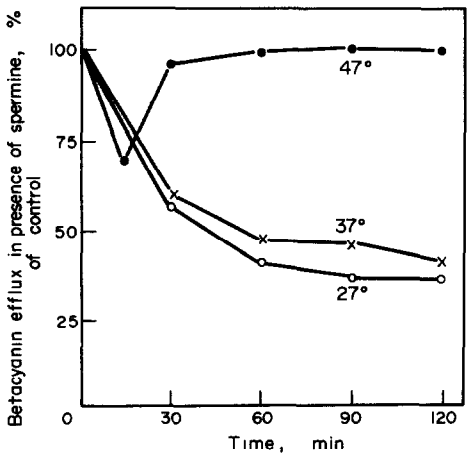

Fig. 2. Effect of temperature on spermine inhibition of betacyanin efflux.

Table 2. Effect of phytate on betacyanin efflux at 37°

Phytate concentration (mM)	Betacyanin efflux (Klett units) at (min) 30	60	90	120
0	115	160	187	245
0.2	120	165	190	235
0.4	127	180	210	238
0.6	110	167	196	236
0.8	110	160	182	216
1.0	95	125	147	175
3.0	85	108	127	155
5.0	77	101	115	133

nucleic acids [14]. We have some evidence (unpublished observations) that polyamines may also bind with phytate, a polyanionic compound. To investigate this further, the effect of phytate on betacyanin leakage was studied. Phytate alone (Table 2) had no effect up to 1 mM but at higher concentrations a slight inhibitory effect was observed. However, when phytate was added together with spermine (Table 3), the inhibitory effect of spermine was reversed by phytate suggesting that phytate must be binding with spermine and thereby effecting the reversal.

Effect of alcohol

Alcohols have been reported to destabilize membranes and thus increase the efflux [7]. Our studies showed that 5–10% ethanol causes a *ca* 3-fold increase in the efflux (Table 4). One consequence of the presence of alcohol suggested by Siegel [11], is that it lowers the dielectric constant of the medium and since the cell surface possesses a variety of charges, lowering of the dielectric constant changes the equilibrium of the system towards the associated state. This lowering of charges would result in greater destabilization of the cations on the cell surface and the dimensional changes in the membrane would result in changed pigment leakage. Since spermine inhibits the pigment leakage it may function like Ca^{2+} ions in lowering the efflux and should then reverse the effect of alcohol. The lower concentrations of spermine in the presence of alcohol were found to decrease the efflux but increasing the concentrations resulted in an increased efflux (Table 5).

Kinetic studies carried out by varying the alcohol concentration at fixed levels of spermine and plotting the data according to Lineweaver and Burk [15] showed (Fig.

Table 3. Reversal of the spermine inhibition of betacyanin by phytate at 37°

Additions	Betacyanin efflux (Klett units) at (min) 30	60	90	120
—	108	185	248	285
Spermine (0.5 mM)	62	86	104	113
Spermine (0.5 mM) + phytate (mM)				
0.1	70	93	104	118
0.4	110	160	200	228
1.0	112	168	238	258

Table 4. Effect of alcohol on betacyanin efflux at 37°

Alcohol (%)	Betacyanin efflux (Klett units) at (min) 30	60
0	171	250
5	180	305
8	268	450
10	365	780

Table 5. Effect of spermine on betacyanin efflux in presence of alcohol at 37°

Additions	Betacyanin efflux (Klett units) at (min) 30	60
—	162	258
Alcohol (8%)	230	470
+ spermine (mM)		
0.2	165	305
0.6	204	425
1.0	250	450
3.0	300	495
5.0	350	540

3) that at 0.2 mM concentration of spermine, the efflux was inhibited competitively while at 1 mM it was almost the same as without spermine. Increasing the concentration of spermine to 5 mM resulted in an activation of efflux. Similarly when the data was plotted according to Dixon [16], the K_i for spermine in the absence of alcohol was 0.6 mM (Fig. 4). The presence of varying concentration of alcohol, however, produced curved lines as a result of both inhibition as well as activation. Based on the

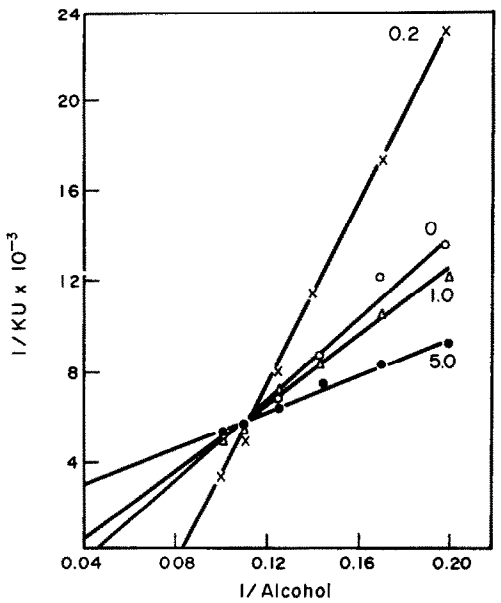

Fig. 3. Lineweaver–Burk plot of betacyanin efflux at varying concentrations of alcohol in the absence and presence of spermine. Spermine concentration in mM is indicated along the lines. KU = Klett units.

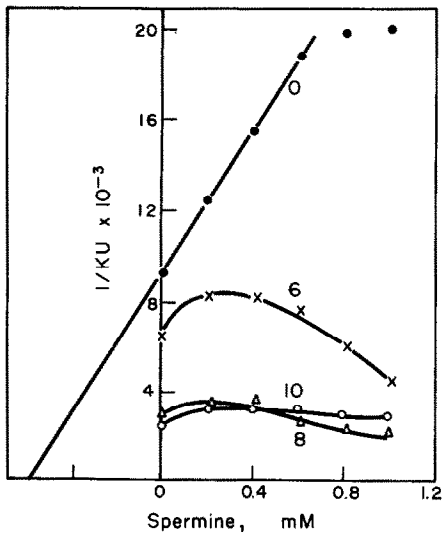

Fig. 4. Dixon plot for the effect of spermine in presence of fixed levels of alcohol. Alcohol concentration (%) is indicated along the lines. KU = Klett units.

mechanism suggested by Siegel [11] for the effect of alcohol and Ca^{2+} ions on the efflux, our results would suggest, that in the presence of alcohol, spermine at low concentrations acts like Ca^{2+} ions in stabilizing the membrane but having no significant effect on the dielectric constant of the medium, whereas at higher concentrations the activating effect may be due to the reversal of the decreased dielectric constant and/or a further distortion of the membrane produced by spermine.

EXPERIMENTAL

Beet root (*Beta vulgaris* L.) was obtained fresh from a local farm. Discs 1–2 mm thick and 10 mm dia cut from the storage tissue were washed twice with running H_2O, then for 1 hr with 4 changes of dist H_2O. 10 discs were transferred to stoppered conical flasks containing 10 ml of H_2O or test soln and incubated at the desired temp.

The betacyanin efflux was followed by measuring the pigment leaked out in the ambient soln at 540 nm. The results, expressed as Klett units, are means of at least 2 independent expts, carried out on the same batch of discs.

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