Plant Physiol. (1988) 86, 441–446
0032-0889/88/86/0441/06/$01.00/0

Utilization of Putrescine in Tobacco Cell Lines Resistant to Inhibitors of Polyamine Synthesis[1]

Received for publication August 1, 1987 and in revised form September 1, 1987

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ABSTRACT

Three tobacco cell lines have been analyzed which are resistant to lethal inhibitors of either putrescine production or conversion of putrescine into polyamines. Free and conjugated putrescine pools, the enzymic activities (arginine, ornithine, and *S*-adenosylmethionine decarboxylases), and the growth characteristics during acidic stress were measured in suspension cultures of each cell line. One cell line, resistant to difluoromethylornithine (*Dfr1*) had a very low level of ornithine decarboxylase activity which was half insensitive to the inhibitor *in vitro*. Intracellular free putrescine in *Dfr1* was elevated 10-fold which was apparently due to a 20-fold increase in the arginine decarboxylase activity. The increased free putrescine titer was not reflected in an increased level of spermidine, spermine, or putrescine conjugation. *Dfr1* cultures survived acidic stress at molarities which were lethal to wild type cultures. Two other mutants, resistant to methylglyoxal bis(guanylhydrazone) (*Mgr3*, *Mgr12*), had near normal levels of the three decarboxylases and normal titers of free putrescine, spermidine, and spermine. Both mutants however had elevated levels of conjugated putrescine. *Mgr12* had an increased sensitivity to acidic medium. These results suggest that increased levels of free putrescine production may enhance the ability of tobacco cells to survive acid stress. This was supported by the observation that cytotoxic effects of inhibiting arginine decarboxylase in wild type cell lines were dependent on the acidity of the medium.

A variety of stressful growth conditions result in the accumulation of free putrescine in many plants. In K^+-deficient plants, plants using only NH_4^+ as a nitrogen source, osmotically stressed or acid stressed plants, increased arginine decarboxylation yielding free putrescine has been observed (6–8, 11, 25, 28). Little is known about the response of cell cultures to the same kinds of stress with respect to the production and utilization of free putrescine. Although variant cell lines with altered polyamine metabolism have been described (13, 16) and partially characterized (13, 17), these cell lines have yet to be used to determine if increased putrescine production can enhance cell survival under stress.

Analysis of the utilization of polyamines in variant suspension cultures may give clues about the significance of the different forms of polyamines which are known to exist in plants. In tobacco callus cells, the conjugated form of the polyamines, especially putrescine, are far more abundant than the free form (17, 22, 27). Although much is known about the developmental regulation of polyamine conjugation (18–22), little is known about the metabolic regulation of conjugation or its physiological significance.

In previous reports (13, 17), the abundance of free and bound polyamines and the enzymic activities of MGBG[2]-resistant cell lines were measured as a first step toward understanding the basis of resistance. It was found that these mutants fall into several distinct classes on the basis of their biochemical characteristics. Some of the cell lines have altered enzymic activities and others have elevated levels of some of the polyamines and polyamine conjugates. The results of a limited genetic analysis suggest that MGBG resistance in some of the cell lines is a nuclear dominant trait which displays linkage with the developmental abnormalities previously described (15). In order to further understand the possbile significance of the regulation of polyamine metabolism we have analyzed the production and conjugation of putrescine in suspension cultures during growth in low pH medium. The results suggest that variant cell lines with increased arginine decarboxylation and high levels of free putrescine production can survive acidic growth conditions better than the wild type counterpart. High levels of conjugated putrescine were associated with diminished survival rates in acidic medium. In addition, inhibition of arginine decarboxylase in wild type cell lines resulted in culture death only at low pH. Sensitivity to arginine decarboxylase inhibition coincided with the accumulation of putrescine during the response to acid stress.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. Wild type cell cultures were initiated from diploid *Nicotiana tabacum* cv xanthi leaves and grown on basal salts medium with 100 mg/L inositol, 10 mg/L thiamine HCl, 1 mg/L pyridoxine HCl, 1 mg/L nicotinic acid (9), 30 g/L sucrose, 0.5 mg/L 2,4-dichlorophenoxyacetic acid (MS 1). The growth rate of liquid suspension cultures and cell viability was measured as previously described (14).

Enzyme Assays. Cells were collected by vacuum filtration, weighed, and then homogenized by sonication (Branson Sonifier, setting 2 with micro tip, 30 s, 3 times) at a ratio of 1 mL homogenization buffer (40 mM sodium phosphate, 10 mM EDTA, 5 mM DTT, 0.04 mM pyridoxal phosphate, pH 7.5) per fresh weight gram of cells. The homogenate was clarified by centrifugation at 10,000*g*. 200 μl of a 10,000*g* supernatant was first spun dialyzed through Sepharose CL-6B (Sigma) at a ratio of 200 μl homogenate per 0.5 mL of packed Sepharose beads

[1] Supported in part by a Damon Runyon-Walter Winchell Cancer Fund Fellowship to A. C. H. (DRG-808), United States Department of Agriculture Grant No. 86-CRCR-1-2057, National Science Foundation grants NSF-DMB-85-44021 (Metabolic Biology), and NSF-DCB-85-00172 (Developmental Biology).

[2] Abbreviations: MGBG, methylgyoxal bis(guanylhydrazone); DFMA, difluormethylarginine; DFMO, difluoromethylornithine; PCA, perchloric acid.

equilibrated in homogenization buffer. Centrifugation was for 2 min at 2000*g* and was performed immediately before the assay. The eluate was added to 10 μl of labeled substrate; the reaction tube was sealed with a paper filter fitted inside a screw top. The filter was spotted with 30 μl of methylbenzythonium hydroxide (Sigma) just prior to the start of the reaction. Reactions were allowed to proceed at 37°C for 15 min (V_{max} conditions) whereupon the reaction was stopped by the addition of 100 μl of 1 M KH_2PO_4. The tubes were resealed and allowed to incubate at room temperature for 2 h. Each filter was then counted in Aquasol 2 (New England Nuclear). Nonenzymic decarboxylation was consistently 5 to 10% of experimental values. [1-^{14}C]Ornithine (58.9 mCi/mM) and *S*-adenosyl[1-^{14}C]methionine (57.6 mCi/mM) were from New England Nuclear; [U-^{14}C]arginine (336 mCi/mM) was from Amersham.

Extraction and Analysis of Polyamines. Cells were harvested by vacuum filtration and homogenized by sonication at 300 mg fresh weight/ml cold 10% perchloric acid (Branson sonifier, setting 2, 30 s, three times). After 30 min on ice the extracts were centrifuged for 10 min at 30,000*g*. The pellet was resuspended in perchloric acid; theee aliquots (200 μl each) of the supernatant and the resuspended pellet were sealed in glass ampoules with 200 μl 12 N HCl. After 18 h at 110°C, the hydrolysates were dried under a stream of air at 85°C then resuspended in 200 μl 10% PCA. Replicates of the nonhydrolyzed supernatant (PCA-soluble, unconjugated polyamines), the hydrolized supernatant (PCA-soluble, conjugated and unconjugated polyamines), and the hydrolyzed resuspended pellet (PCA-insoluble, conjugated polyamines), were dansylated and chromatographed according to the procedures of Flores and Galston (5). Soluble conjugate levels were determined by subtraction of the unconjugated value from the total hydrolysate. Extracellular polyamine was determined after clarifying the medium from late log phase cells at 3000*g*. Typically, 400 μl of medium was dansylated for polyamine analysis. Dry weight was determined after baking suspension cells in a vaccuum oven at 80°C overnight. Dansyl polyamines were visualized by UV fluorescence and quantified by fluorimetry (Perkin-Elmer model LS3 spectrofluorimeter at 350 nm excitation, 495 nm emission) after scraping the polyamine bands into 2 ml of ethyl acetate.

RESULTS

Decarboxylase Activities and Morphology of Cell Lines Resistant to MGBG or DFMO. Two cell lines used in this study were selected for resistance to 10 mM MGBG and have been propagated as liquid suspension cultures as previously described (14). An additional cell line was selected for resistance to 10 mM DFMO (*Dfr1*) using the same method of ultraviolet mutagenesis as was used to isolate the MGBG-resistant lines (14). Each callus culture was adapted to growth in liquid medium over a period of 1 month. Growth rates of the resulting suspension cell lines were approximately the same as wild type suspension cells. The cell morphologies, however, were distinctly different from wild type. In general, a high percentage of cells were smaller and elongated with less vacuolization. This was especially true of the *Dfr1* cells which were uniformly smaller and very elongated (Fig. 1). This is in contrast to the results described by Berlin and Forsch (3) where exposure of tobacco cells to DFMO led to cellular expansion. The smaller cell size of *Dfr1* cultures was reflected in a 4-fold increased yield of protein per g fresh weight compared to wild type cells. On a dry weight basis *Dfr1* cultures had the same amount of protein as wild type.

One of the MGBG-resistant cell lines, *Mgr12*, had normal levels of arginine, ornithine, and *S*-adenosylmethionine decarboxylases (Table IA). The *S*-adenosylmethionine decarboxylase activity, however, was 50% resistant to MGBG at concentrations ranging from 1 to 10 mM (17). The other MGBG resistant cell line, *Mgr3*, also had normal levels of the decarboxylases. However, the *S*-adenosylmethionine decarboxylase activity in *Mgr3* was completely inhibited by MGBG at concentrations above 100 μM (17). *Dfr1* had normal levels of *S*-adenosylmethionine decarboxylase but had low levels of ornithine decarboxylase activity. In the absence of DFMO, the ornithine decarboxylase activity from *Dfr1* was about 10% of wild type on the basis of *in vitro* measurements; in 1 to 10 mM DFMO, the activity was consistently 5% of wild type indicating that half of the residual activity was insensitive to the inhibitor. *Dfr1* cells contained an elevated level of arginine decarboxylase activity which was 20-fold higher than in wild type cultures. By enzyme assay, total decarboxylation leading to putrescine in *Dfr1* was therefore twice as high as wild type.

Polyamine Levels in Resistant Cell Lines. Total polyamines were measured in three fractions as follows: (a) the free polyamines, soluble in 10% perchloric acid, (b) soluble conjugates, detected only after treating fraction 1 with 6 N HCl at 110°C for 18 h, (c) insoluble conjugates, released from the resuspended perchloric acid pellet by hydrolysis. The values for the soluble conjugates (fraction 2) were obtained after subtracting the free polyamines (fraction 1) from the total polyamine in the hydrolysate. In general, little difference between wild type and resistant suspension cell lines was detected when comparing free spermidine or spermine levels. The putrescine pools differed dramatically among the cell lines (Table IB).

Both *Mgr3* and *Mgr12* contained normal levels of free putrescine but elevated levels of the acid soluble putrescine conjugates. *Mgr12* also contained elevated levels of the insoluble putrescine conjugates as well. Very elevated levels of free putrescine were found in *Dfr1* which were not reflected in equivalent levels of conjugated putrescine. The three cell lines therefore contained elevated levels of either free putrescine (*Dfr1*), soluble conjugated putrescine (*Mgr3*), or total conjugated putrescine (*Mgr12*).

An additional unusual characteristic of *Dfr1* cultures was the high level of free putrescine found in the growth medium (Table IC). In general, in each cell line, the free putrescine levels found in the medium was proportional to the intracellular free putrescine. *Dfr1*, therefore, had a 10-fold excess of both intracellular and extracellular putrescine.

Stress Response of Resistant Cell Lines. With the exception of *Dfr1*, all of the cell lines, when exposed to low pH, had increased levels of arginine decarboxylase and increased levels of putrescine both intracellular and in the growth medium. On average, wild type, *Mgr3*, and *Mgr12* displayed approximately 7-fold increases in arginine decarboxylase giving rise to increases in free putrescine titer of about 3-fold. *Dfr1* had a slight increase in arginine decarboxylation as well as a 20% increase in the abundance of both intracellular and extracellular free putrescine at low pH. With the exception of *Mgr3* the conjugated levels of putrescine in all the cell lines in response to low pH were unchanged. In *Mgr3* the soluble putrescine conjugates decreased almost 3-fold after the pH shock; the insoluble conjugate levels were unchanged.

Growth Characteristics of Resistant Cell Lines. Since the resistant cell lines contained increased levels of either free or conjugated putrescine, growth characteristics of these cell lines at low pH might indicate which, if any, of these molecular forms of putrescine is significant during a stress response. In these experiments, cultures were exposed to 6 or 15 mM citric-Tris (pH 3.5). *Mgr3* had the same growth rate as wild-type in 6 mM citrate, whereas *Mgr12* had a slower growth rate at both the low and high ionic strengths (Table II). *Dfr1* cultures were unusual in that they grew well in 15 mM citrate. Morbid staining after the high ionic strength pH shock showed that approximately 90% of the wild type cells were killed within 24 h (see below) whereas approximately 75% of *Dfr1* cells survived. The results suggest

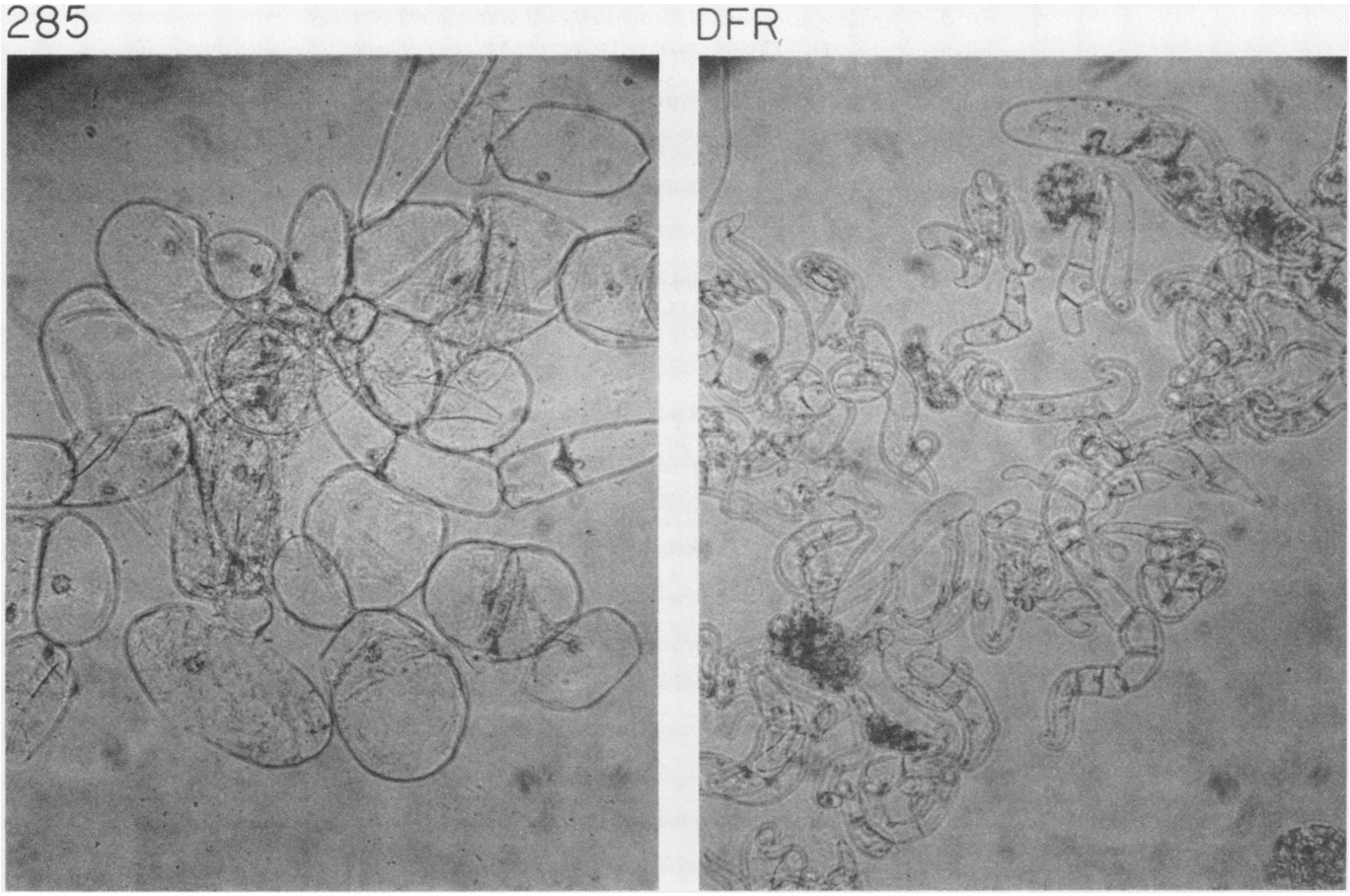

FIG. 1. Morphology of DFMO-resistant cells. Log phase suspension cells were photographed at a 250-fold magnification. The wild type cells are labeled 285, referring to the original date of propagation in culture. The DFMO-resistant cells are labeled DFR. The two photographs are presented at the same magnification.

that in this cell line increased levels of arginine decarboxylation and free putrescine are associated with an enhanced ability to survive acidic shocks. In *Mgr12*, increased levels of total conjugated putrescine are associated with a diminished ability to tolerate the low pH medium. A comparison of the rate of neutralization of the media after addition of citrate revealed no difference between *Dfr1* and wild type. Apparently, the elevated level of secreted putrescine in *Dfr1* is not contributing to a more rapid neutralization of the medium.

Inhibition of Arginine Decarboxylase is Cytotoxic at Low pH. Wild type cultures were challenged for 7 d with increasing ionic strengths of pH 3.5 citrate in the presence and absence of 10 mM difluoromethylarginine (DFMA, the suicide inhibitor of arginine decarboxylase [10]). Half of the cultures without DFMA were harvested after 24 h to measure the putrescine levels. The remainder were left for 7 d to measure growth. At 6 mM citrate or higher, cultures which would have ordinarily recovered from the acid stress did not grow at all in the presence of DFMA (Fig. 2). Six mM was apparently the minimum ionic strength of acidity which was lethal after 7 d in the presence of DFMA. In the absence of DFMA, 6 mM citrate elicited a nearly maximal increase in the free putrescine levels. In these experiments, only the initial accumulation of putrescine was measured (*i.e.* after 24 h). There was no effect on the levels of spermidine or spermine. The long term effects after 7 d of the low pH shock was not quantified since a large percentage of the cells were dead whereas after 24 h greater than 90% of the cells were still viable as judged by dye exclusion (14).

After the addition of citrate, neutralization of a 50 ml culture (2×10^6 cells) occurred at a constant rate of 0.17 to 0.25 pH units per hour. Cultures were neutralized to pH 6 within 12 h. Cells responding to the acidic shock in the presence of DFMA neutralized the medium at the same rate as cells which were not exposed to the inhibitor. After 36 h, in cultures without DFMA, arginine decarboxylase levels and free putrescine were still maximally elevated suggesting that maintenance of the high enzymic activity and elevated titer of free putrescine was not strictly linked to the external pH. In a related experiment, cell were exposed to the low pH shock for varying periods of time followed by neutralization of the medium to pH 6.0 with 2 M Tris-Cl (pH 8.0). Arginine decarboxylase and putrescine levels were then measured 24 h after the acidic shock to estimate the minimum period of low pH exposure necessary for induction of arginine decarboxylase activity. A 4 h exposure to low pH buffer is required to elicit a maximal increase in arginine decarboxylase for at least 36 h (data not shown). Exposure of cells to low pH shock for as little as 30 min resulted in a 50% increase in putrescine titer after 36 h. Apparently, the low pH treatment served to trigger a long term response which was required for cell survival even after the medium was neutralized.

DISCUSSION

There currently is no encompassing hypothesis to account for the accumulation of putrescine and other polyamines under stress. A number of possibilities have been suggested including substitution for divalent ions in the regulation of nucleic acid function (3), maintenance of the cellular ionic balance (1, 4), and maintenance of the cellular pH (26). A common denomi-

Table I. *Decarboxylase Activities and Putrescine Levels in Resistant Cell Lines*

Stationary phase tobacco suspension cells resistant to either DFMO (*Dfr1*) or MGBG (*Mgr3*, *Mgr12*) were diluted 4-fold into medium containing citrate-Tris pH 6.0 or pH 3.5 at a concentration of 6 mM citrate. After 36 h an aliquot of the cells was taken for measurement of the free and bound putrescine titer and the decarboxylase activities. In (A), the arginine (ARG), ornithine (ORN), and *S*-adenosylmethionine (SAM) decarboxylase activities are shown as a function of the pH of the growth medium. The activities in parentheses are the values obtained in the presence of 1 mM difluoromethylornithine (*Dfr1*) or 1 mM methylglyoxal bis(guanylhydrazone) (*Mgr3*, *Mgr12*). In (B), the abundance of free and bound putrescine in cells exposed to 6 mM citrate is shown. In (C), free putrescine in the growh medium from each culture is shown. The results are expressed as the mean ± SE for three or four determinations.

	pH 6.0			pH 3.5		
(A)			*pmol CO_2/mg protein/h*			
	ARGdc	ORNdc	SAMdc	ARGdc	ORNdc	SAMdc
Control	35.5 ± 4.2	57.3 ± 6.0	23.4 ± 1.9	314 ± 50.6	50.8 ± 8.2	20.7 ± 3.1
Dfr1	653 ± 78.8	5.1 ± 0.8 (2.3 ± 0.6)	19.7 ± 2.3	721 ± 80.5	4.5 ± 0.6	21.2 ± 2.9
Mgr3	36.2 ± 5.3	52.9 ± 4.9	25.8 ± 3.2 (0)	290 ± 28.8	45.6 ± 6.3	18.4 ± 3.0
Mgr12	44.7 ± 6.1	49.0 ± 6.2	30.1 ± 3.3 (14.1 ± 2.2)	255 ± 30.2	40.6 ± 5.8	27.7 ± 3.9
(B)			*nmol putrescine/mg dry weight*			
	S[a]	SH[b]	PH[c]	S[a]	SH[b]	PH[c]
Control	4.0 ± 0.4	25.1 ± 3.0	13.6 ± 1.7	14.2 ± 1.3	26.7 ± 3.2	12.5 ± 1.9
Dfr1	41.2 ± 4.6	16.9 ± 2.1	21.2 ± 3.1	50.1 ± 6.8	16.4 ± 2.8	20.9 ± 2.7
Mgr3	6.1 ± 0.5	70.6 ± 8.4	16.7 ± 2.9	18.8 ± 3.1	28.2 ± 4.0	18.0 ± 2.8
Mgr12	5.1 ± 0.7	82.4 ± 9.0	41.0 ± 5.6	15.3 ± 1.7	92.1 ± 9.8	44.6 ± 6.0
(C)			*Putrescine in the Growth Medium, nmol/ml*			
Control		0.9 ± 0.2			3.15 ± 1.0	
Dfr		8.3 ± 1.0			10.0 ± 1.5	
Mgr3		1.0 ± 0.2			3.7 ± 0.5	
Mgr12		0.8 ± 0.1			3.5 ± 0.6	

[a] PCA-soluble fraction of putrescine. [b] PCA-soluble conjugated fraction of putrescine detected after acid hydrolysis. [c] PCA-insoluble conjugated fraction of putrescine detected after hydrolysis.

Table II. *Growth Characteristics of Resistant Cell Lines*

Stationary phase tobacco suspension cells resistant to either DFMO (*Dfr1*) or MGBG (*Mgr3*, *Mgr12*) were diluted 4-fold into medium containing citrate-Tris pH 6.0 or pH 3.5 at a concentration of either 6 or 15 mM citrate. An aliquot of the cells was taken to determine the total dry weight. The remainder of the cells were harvested after 7 d for measurement of the dry weight. The increase in dry weight after 7 d of exposure to either 6 or 15 mM citrate is shown; the results are expressed as the ratio of increase in the dry weight of cultures exposed to pH 3.5 compared to cultures exposed to pH 6.0 citrate.

	Ratio of Increase in g Dry Weight	
	6 mM citrate	15 mM citrate
	pH 3.5/pH 6.0	
Control	0.6	0.05
Dfr1	0.9	0.5
Mgr3	0.5	0.08
Mgr12	0.3	0.01

nator which could relate potassium and magnesium deficiencies, ammonium excess, SO_2 exposure, and acidification, is the production of protons which accompanies these and other stress conditions. The accumulation of protonated putrescine may be a mechanism by which plant cells balance excess H^+. Although putrescine production increases sharply in many plants in response to these stresses, it has been argued that this putative role for putrescine cannot provide an explanation for all stress-induced increases in putrescine (28). One example might be osmotic stress where the concentrations of proline and glycine betaine are often far in excess of the induced putrescine level. It was suggested that if putrescine is important in resistance to ionic stress it might differ from the other compounds by affecting microenvironments around macromolecules and cellular structures (28). Also, changes in the intracellular pH associated with osmotic stress may be an important factor in the osmotic induction of putrescine synthesis. In this regard, it is well established that stromal acidification is a mediating mechanism involved in the inhibition of photosynthesis during osmotic stress (2).

We have been interested in the biochemical characterization of cell lines which are resistant to inhibitors of polyamine synthesis. Although the levels of polyamines in these and other cell lines have previously been reported (12, 16), we have extended these studies to determine if these cell lines reveal anything about the role of putrescine during acidic stress adaptation. The cell lines used in this study represent three classes of elevated putrescine: free putrescine in *Dfr1*, soluble conjugated putrescine in *Mgr3*, and total conjugated putrescine in *Mgr12*. Our results suggest that elevation of total conjugated putrescine is associated with increased sensitivity to acid stress whereas elevated free putrescine may enhance cell survival after low pH shock. This provides support for the hypothesis stated above that accumulation of free putrescine may be a mechanism to balance excess intracellular protons.

A major drawback of this type of analysis is the incomplete biochemical characterization of cell lines. There is always the possibility that an unmeasured trait is responsible for stress adaptation. Inhibition of arginine decarboxylation and putrescine production during stress responses of wild type cell lines is therefore an essential corollary to characterization of mutant cell lines. It was found that inhibition of arginine decarboxylase with DFMA lead to an increased sensitivity of wild type cells to low pH. Using ionic strength as a variable, optimum stimulation of putrescine accumulation was coincident with the cytotoxic effects of inhibiting arginine decarboxylase. A possibility which has not been explored is that arginine depletion is occurring by decar-

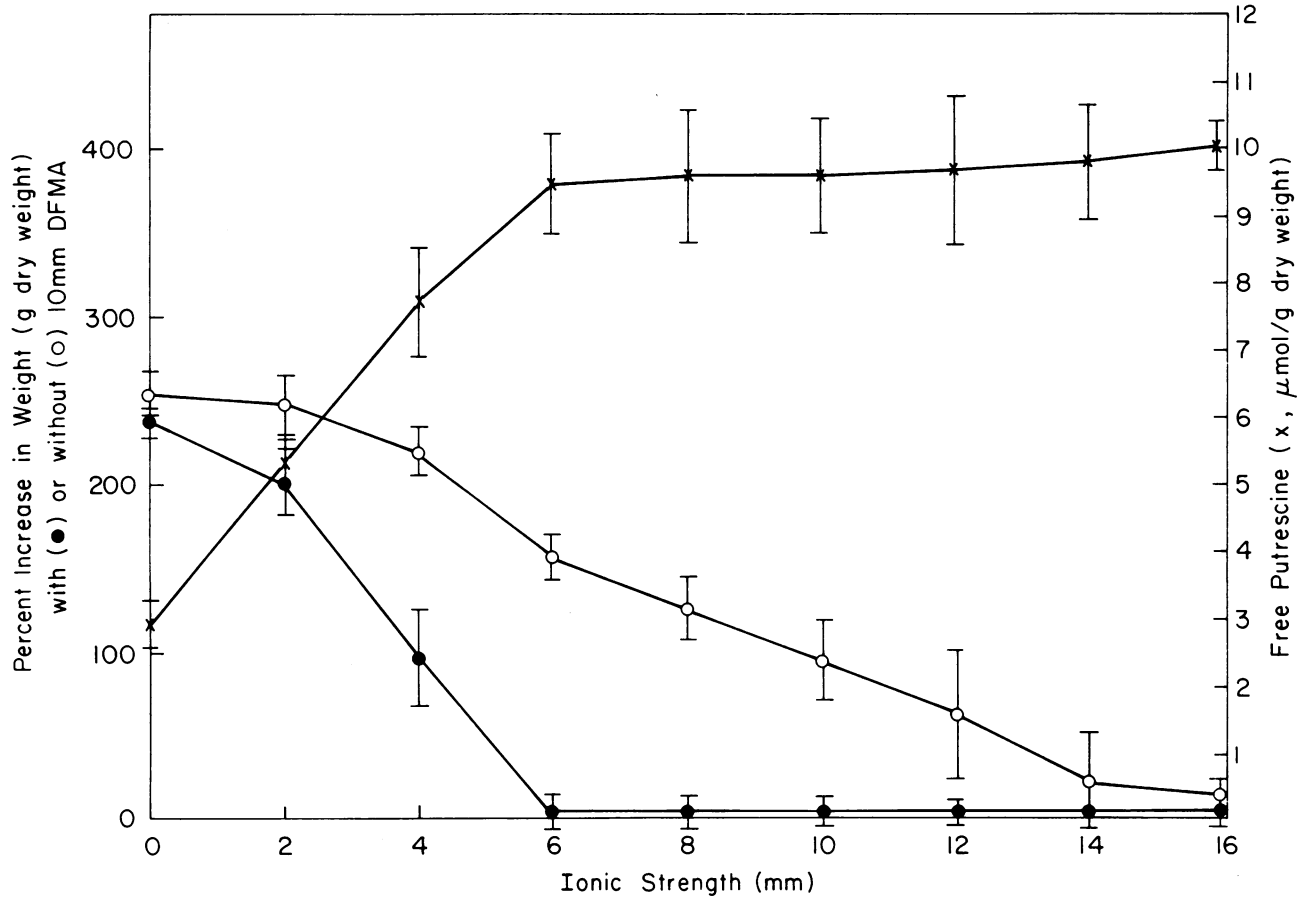

FIG. 2. Effect of difluoromethylarginine and low pH on cell growth. Stationary phase cultures were diluted 4-fold into medium containing citrate-Tris pH 3.5 of varying ionic strengths. An aliquot from each culture was taken to determine the initial dry weight. To half of the paired cultures was added 10 mM difluoromethylarginine. After 24 h an aliquot from each culture containing no difluoromethylarginine was taken to determine the intracellular free putrescine levels. The cultures were further allowed to grow for 7 d and were harvested to determine the increase in dry weight.

boxylation and may be a significant response to this type of stress. By analogy with other decarboxylation reactions which are apparently involved in the regulation of intracellular pH, this possibility is unlikely. For example, generation of CO_2, OH^-, and pyruvate from malate decarboxylation may be a biochemical pH stat that copes with short term fluctuations in cytoplasmic pH (24). Another example is production of α-aminobutyric acid from glutamate decarboxylation which, it has been suggested, can offset excess H^+ production in the absence of compensating H^+ efflux (23). Measurement of arginine pools as well as radiotracer studies with arginine and ornithine precursors should be performed in order to confirm that putrescine production is the primary metabolic effect of arginine decarboxylation under acid stress.

The relatively low levels of putrescine conjugation in *Dfr1* also suggests that the high levels of arginine decarboxylase and free putrescine are not sufficient to induce equivalently high levels of putrescine conjugation. A possible conclusion from our results is that the conjugated putrescine populations are not available for maintaining free putrescine titers. This is suggested by *Mgr12* which had a stress induction of free putrescine accumulation indistinguishable from wild type yet contained high levels of total conjugates. Also, stress treatment of *Mgr3* resulted in a 60% reduction in soluble conjugated putrescine. This diminished conjugation may have been due to an acid sensitive conjugation reaction or an increased degradation of these conjugates during stress. In either case, reduction of conjugate pools was not reflected in an equivalent increase in free putrescine.

The molecular basis of resistance of these cell lines is an area of speculation. One can suppose that the MGBG insensitive SAMdc in *Mgr12* operates *in vivo* providing a kinetic mechanism of resistance. One can also speculate that this might be due to a single mutated allele producing an inhibitor-insensitive enzyme. In addition, the elevated levels of putrescine and spermidine conjugates in *Mgr3* and *Mgr12* may provide some resistance by out-competing the MGBG. In the case of *Dfr1*, only low levels of ORNdc were detected *in vitro*; however, some of this activity was insensitive to DFMO. It was not clear whether this residual activity was reflected *in vivo* or was an artifact of the homogenization and extraction procedure. Tracer studies to monitor the conversion of ornithine to putrescine in *Dfr1* will be helpful in resolving this possibility.

Further analysis of *Mgr3*, *Mgr12*, and *Dfr1* during regeneration is required to determine if their high levels of free or conjugated polyamines persist during whole plant development. It is tempting to speculate that the developmental abnormalities observed in these and other cell lines originate in an inappropriate polyamine metabolism during regeneration. In general, the regeneration phenotypes of cell lines with altered polyamine metabolism has suggested that regulation of the polyamine pathway may be a critical requirement during the development of organized tissues (13, 15). All plants resistant to either MGBG or DFMO were developmentally abnormal (15). The phenotypes ranged from inability to initiate regeneration, arrested development in shoot cultures, dwarf plants, and developmental switches in flowers. Continued isolation of polyamine mutants will enable a

more comprehensive genetic analysis of the polyamine pathway and may reveal the role of these compounds during stress adaptation as well as in higher plant development.

Acknowledgments—The authors would like to thank Patricia Scolnik for her technical help in the early phases of this work, and to also thank Robert Slocum and Irwin Greenblatt for helpful discussions. DFMA and DFMO were obtained courtesy of Merrell-Dow Research, Cincinnati, OH.

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