Review

Polyamines in Plant Physiology

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ABSTRACT

The diamine putrescine, the triamine spermidine, and the tetra-
mime spermine are ubiquitous in plant cells, while other poly-
mines are of more limited occurrence. Their chemistry and path-
ways of biosynthesis and metabolism are well characterized.
They occur in the free form as cations, but are often conjugated
to small molecules like phenolic acids and also to various mac-
romolecules. Their titer varies from approximately micromolar to
more than millimolar, and depends greatly on environmental
conditions, especially stress. In cereals, the activity of one of the
major polyamine biosynthetic enzymes, arginine decarboxylase,
is rapidly and dramatically increased by almost every studied
external stress, leading to 50-fold or greater increases in putres-
cine titer within a few hours. The physiological significance of
this increase is not yet clear, although most recent work suggests
an adaptive, protective role. Polyamines produced through the
action of ornithine decarboxylase, by contrast, seem essential
for DNA replication and cell division. The application of exoge-
nous polyamines produces effects on patterns of senescence
and morphogenesis, suggesting but not proving a regulatory role
for polyamines in these processes. The evidence for such a
regulatory role is growing.

Most references to PAs in plants are less than two decades
old. It is therefore not surprising that their role in the physi-
ology of the plant, if any, is still uncertain. What is known
with reasonable certainty is that (a) the major PAs, putrescine,
spermidine, and spermine, are found in every plant cell in
titers ranging from approximately 10 μM to approximately
millimolar, together with the enzymes regulating their metab-
olism; (b) they occur in the free form or bound to phenolic
acids, other low mol wt compounds or macromolecules; (c)
their titer is very responsive to external conditions, such as
light, temperature, and various chemical and physical stress
agents; and (d) the application of exogenous PAs to plants or
plant parts can produce visible effects such as the prevention
of senescence in excised leaves and the formation of em-

1 We wish to dedicate this article to the memory of an outstanding
scientist and colleague, Ruth L. Satter, who worked in this laboratory
from 1968 to 1980. It was at Yale that she began the work with
pulvini that remained central to all her subsequent investigations.

2 Abbreviations: PA, polyamine; Put, putrescine; Spd, spermidine;
Spm, spermine; ADC, arginine decarboxylase; ODC, ornithine
decarboxylase; DFMA, α-difluoromethylarginine; DFMO, α-difluoro-
methylornithine.

bryoids or floral primordia in certain otherwise vegetative
tissue cultures. These facts compel us to examine the possible
role of PAs as regulators of physiological processes in plants.
While the demonstration of correlations between PA titer
and a physiological response does not establish a causal con-
nection between the two, such activity is usually a necessary
prelude to providing such proof. This has certainly been the
initial procedure through which the now accepted plant hor-
mones have been recognized and validated. PA research now
seems poised to move from the phase of establishing correla-
tions to the delineation of processes and specific molecular
mechanisms through which control is exerted. All in all, the
present evidence suggesting a regulatory role for PAs seems
not dissimilar from that for the recognized plant hormones at
a similar stage in their history.

A BRIEF HISTORICAL PERSPECTIVE

As pointed out by Seymour Cohen in his provocative 1971
monograph (6), the history of PA biochemistry goes back
more than 300 years. Antoni van Leeuwenhoek, staring at
human semen through the lenses of his primitive microscope
in 1678, noted the deposition of stellate crystals in aging
sperm. More than 200 years later, the basic component of
these phosphate crystals was named spermine, after the
source, but it was not until the middle 1920s that its correct
chemical composition and structure were determined. Sper-
midine was also discovered and named at about this time.
PAs remained interesting mainly to chemists for about the
next half century, when Cohen’s book, based on a series of
invited lectures at the Collège de France, directed attention
to the possible biological importance of these compounds
and stimulated research in many areas, including plant physi-
ology. Among his provocative generalizations was the observation
that modern biochemistry has been concerned mainly with
anions and has tended, at least until very recently, to neglect
cations, of which the PAs are one of the cell’s major organic
representatives.

Work in the physiology and biochemistry of PAs in plants
has been centered in only a few laboratories. The Long Ashton
research station of the University of Bristol has been im-
portant since the 1950s, culminating in definitive work on plant
polyamine biochemistry by Terence Smith. A 1973 paper by
Nello Bagni and his wife Donatella Serafini-Fracassini of the
University of Bologna (4), delivered at a plant growth hor-
monesymposium in Tokyo, was probably the first to suggest
that polyamines have regulatory action in plants. A subse-
quent symposium in this series (23) included, for the first
time, a group of papers on PAs, and since then, research
in this subject has spread to many countries of the world. Recent
reviews of PAs in plants (3, 8, 9a, 12, 21) give comprehensive
coverage of the literature up to about 1988, while another
recent review summarizes the situation in animals and micro-
organisms as well (22). International biennial meetings are
summarized in such series as Advances in Polyamine Research
(1) and similar publications (20, 24).

THE ROLE OF PAs IN BIOLOGY

The essentiality of PAs for both prokaryotic and eukaryotic
microorganisms has been established. For example, Haemo-
ophilus parainfluenzae will not grow on a synthetic medium
without the addition of either putrescine (Put), spermidine
(Spd), spermine (Spm) or 1,3-diaminopropane, a product of
Spd and Spm oxidation (6). In both Escherichia coli and
Saccharomyces cerevisiae, a series of single gene mutants is
available for most of the steps in the biosynthetic chain for
PAs (22). In E. coli, a mutant with an absolute requirement
for PAs suffers from a lesion in a ribosomal protein, which is
presumably corrected by the addition of a PA. In Saccharo-
myces, where Put can arise only from ODC action, several
mutants at the ODC locus fail to grow in the absence of added
PAs. In addition, mutants unable to convert Put to the higher
PAs Spd and Spm are unable to sporulate or to support the
replication of a double-stranded RNA ‘killer factor.’ These
results indicate that polyamines are not only essential for the
growth of this organism, but that, under appropriate condi-
tions, specific polyamines may exert specific morphogenetic
control functions as well. This intriguing possibility has sug-
gested several recent experiments on plants, detailed below.
Other microorganisms with established polyamine-requiring
mutants include Neurospora crassa and Aspergillus nidulans
(6). In some thermophilic bacteria, the organism produces
unusual, long chain polyamines in response to the stressful
condition of elevated temperature (Oshima, in ref. 3). These
compounds apparently play a role in the protection against
inactivation of enzymes that are especially sensitive to high
temperature.

There are no similar clearly defined mutants showing abso-
luute requirement for PAs in either animals or plants, but
inferences about PA essentiality for particular processes have
flowed from the use of specific, enzyme-activated ‘suicide
inhibitors’ for both ODC and ADC. If the hydrogen on the
α-carbon of either arginine or ornithine is substituted by a
difluoromethyl-substituent, the resulting compounds, DFMA
and DFMO, combine irreversibly with the active sites of ADC
and ODC, respectively, thus preventing further enzyme activi-
ty. Treatment with either or both (or similar, less specific
inhibitors) can result in depletion of one or more of the PAs
in the cell. If such treatment results in inhibition of some
cellular process, and if this inhibition is reversible by PA
application, then it is reasonable to infer that PAs participate
in that cellular process. For example, it has recently been
shown that in rice plants exposed to anaerobic conditions,
Put formation from arginine by ADC action is greatly in-
creased. The elongation of rice coleoptiles under such condi-
tions is closely related to the Put titer, but is relatively unre-
sponsive to auxin. If ADC action is blocked by DFMA, then
Put titer and coleoptile elongation are diminished; if Put is
then added, both inhibitions are reversed. It appears, from
these experiments, that Put is required for the anaerobic
eelongation of rice coleoptiles (15). Interestingly, Put has no
effect on aerobic elongation of rice coleoptiles, where auxin
is active.

By the use of similar indirect techniques, it has been shown
that PAs are required for the completion of cell division in
some animal and higher plant cells (3). Frequently, inhibitor-
treated cells are locked into the G1 stage of the cell cycle, but
they progress to the S phase when PAs are added. This has
led to the opinion that PAs may be required for DNA repli-
cation. This fact, together with the extraordinarily high turn-
over number for ODC, its rapid and massive activation by
growth stimulants, and its high activity in metastasizing tu-
mors, has greatly interested oncologists. In fact, DFMO was
originally synthesized by the Merrell laboratories as an anti-
cancer drug, a role in which it is partially effective when given
together with other substances (1).

At cellular pH values, PAs are cations, and it has been
frequently proposed that at least a part of their action results
from association with anionic cellular macromolecules such as
DNA, RNA, phospholipids, or certain proteins (6). Cer-
tainly there are many reported instances in which an exoge-
nous labeled PA becomes bound to a cellular fraction from
which it can be released only by DNAase or protease. There
are also reports in which PA attachment to membranes by
way of phospholipids results in altered patterns of solute
permeation through those membranes. It has been demon-
strated that PAs are major cationic components of T4 bacte-
riophage and other viruses, together with calcium and mag-
nesium, and can be used to “package” DNA and RNA more
compactly for introduction into receptor cells. PAs are also
tenaciously bound by ribosomes, and in vitro can be used to
reassociate 50S and 30S ribosomal subunits into approxi-
mately 70S particles (1).

In addition to their role as cations, presumably associated
with anions by means of electrostatic charges, PAs can also
be covalently attached to glutamic acid constituents of pro-
teins through the action of transglutaminases and perhaps
other enzymes (Beninati and Folk, in ref. 24). Specific poly-
amine-binding proteins, presumably formed through this
mechanism, have been found in various types of cells, includ-
ing plants (2).

ARGININE DECARBOXYLASE AS A GENERAL ‘STRESS
ENZYME’ IN CEREALS

In 1952, Richards and Coleman (17) discovered that barley
plants grown in hydroponic culture responded to suboptimal
potassium levels by the accumulation of high titers of Put.
Since then, Put accumulation, especially in cereals, has been
shown to occur in response to such varied stresses as water
derprivation, high external osmolarity, high external concen-
trations of ammonium or hydrogen ion, deficiency or excess
of other monovalent cations, atmospheric pollutants such as
sulfur dioxide and cadmium ion, low temperature in subtrop-
ical species, and anaerobiosis. In all cases where the pathway of extra Put formation has been investigated, ADC activation has been implicated. It thus appears valid to refer to ADC as a general stress enzyme in cereals, and to Put accumulation as a general sign of stress-induced ADC activation in this group of plants (9, Galston in ref. 3).

The accumulation of Put begins very rapidly after the application of stress (9). For example, oat leaves show some Put accumulation and ADC activation within 1 to 2 h after exposure to a hypertonic sorbitol solution. Cycloheximide can inhibit this process if it is applied within the first hour after the application of stress, but not later. In some experiments, this inhibitor had to be applied within several minutes to have full effect. These results indicate that protein synthesis is required for the increase in ADC activity to be manifest, and that such synthesis is turned on rapidly after stress application. The fact that DFMA is also an effective inhibitor, but that DFMO is not, suggests that ADC synthesis is activated during stress. This hypothesis has received extra support from some experiments involving [35S]methionine labeling of purified oat leaf proteins in control and stressed leaves (Young and Galston, in ref. 20).

**PAs AND SENESCEENCE**

Polyamines, especially Spd, are generally abundant in young, nonsenescent organs, and decline to a lower titer as organs age and senesce (11). The decline in PA titer seems to approximate the pattern of appearance of the symptoms of senescence, as well as the decline in ADC activity. Since the addition of exogenous PAs prevents the appearance of the symptoms of senescence, it seems valid to postulate that the onset of senescence may be caused by a decline in ADC activity and therefore of PA titer. Many publications have examined this hypothesis; most are supportive, but some are contradictory.

The antisenesceence action of exogenously applied PAs was first noted in freshly prepared mesophyll protoplasts of oat and other cereals. Their rapid decline in ability to incorporate leucine into protein and uridine into RNA was arrested by PAs applied in the 10–100 μM range. The exogenous PAs also retarded Chl breakdown in the protoplasts, while enhancing their ability to synthesize DNA and to undergo several rounds of division. Later, similar effects were noted in leaves, where exogenous PAs retarded or arrested completely the abnormal immediate rise in RNAase activity following leaf excision. After about 6 to 8 h, the exogenous PAs also retarded and diminished a rise in acid protease activity, and after 24 to 36 h, they diminished the rate at which Chl disappeared. All these results might be associated with an antiethylene action, since applied PAs were shown to inhibit ethylene production in vivo as well as the effectiveness of applied ethylene (11).

In the experiments with excised, peeled, oat leaf segments, it was found that the exogenous PA was not required for the entire 48 to 72 h period of senescence. In fact, as little as 30 to 60 min exposure to 0.1 to 1.0 mM Spm sufficed to prevent the development of symptoms of senescence which normally occurred up to 36 h later. It is thus clear that very little PA can affect major metabolic processes over a long period, a characteristic one usually associates with oligodynamic, regulatory molecules. During the 30 to 60 min of contact between leaf and PA, calcium, itself a repressor of senescence in some systems, interfered with the effect of the applied PA. Since calcium did not interfere when given before or after the PA, it is inferred that calcium interferes with the entry of the PA into the cell, possibly by competing for a common entry site.

Tomato fruits also show evidence of the antisenesceence action of PAs, which might result from PA-ethylene interactions. Alcobaca and other varieties of tomato with extended shelf lives due to retardation of senescence have higher than usual titers of Put, which shows an increase rather than the usual decline during ripening (7). In Liberty, another variety of tomato, an unusual increase in Put during ripening is correlated with an unusually low production of ethylene and an increased storage life (19). On the other hand, there are also examples of poor correlations between PAs and ethylene, or PAs and ripening patterns. This could result from the fact that senescence, like ethylene production and action, can be controlled by more than one stimulus or pathway, especially in different plant systems. However, some workers have suggested that these PA effects on senescence are artifacts. According to this view, exogenous PAs are nonspecifically toxic, and thus prevent senescence by interfering with the formation of enzymes essential to the synthesis of ethylene. The decreased fluidity of membrane components in PA-treated cells is taken as evidence for this point of view (18).

**PAs AND MORPHOGENESIS IN PLANTS**

Consideration of PAs as potential regulators of morphogenesis in plants flows from their demonstrated effects in other organisms. Probably the most suggestive is the already-cited work of C. W. Tabor (22) with the yeast *Saccharomyces cerevisiae*. Tabor was able to obtain single gene mutants blocked at several points in the biosynthetic pathway for PAs. A mutant blocked at the ODC locus was unable to synthesize Put or any other PA and failed to grow at all unless Put or other PA was added to the medium. This shows that PAs are required and that the only pathway for their synthesis in this organism is by way of ODC. If the mutation is at the Spd synthase locus, then the organism can grow but never sporulate. This shows that Put alone can support growth, but that a higher PA (Spd or Spm) is required for the differentiation of specialized sporangial cells. In the absence of these higher PAs, a double-stranded RNA ‘killer factor’ also failed to replicate, again indicating a possible connection between PAs and nucleic acid biosynthesis.

We were able to build on this observation in studies with various phytopathogenic fungi (Galston and Weinstein in ref. 24). Using the ‘suicide inhibitor’ DFMO to block ODC, we were able to show that depletion of PAs led to an inhibition of growth that was reversible by the addition of exogenous PAs to the medium. In several of the Ascomycete and Basidiomycete species examined, the inhibited cultures also failed to initiate sporangia, thus generalizing the yeast results to other fungi. It occurred to us that inhibition of sporulation in phytopathogenic fungi might be useful in the protection of
crop plants against infection. Higher plants, possessing both the ADC and ODC pathways for the formation of Put, should not be unduly inhibited by DFMO, but at least some fungi, depending entirely on ODC, should have both their growth and sporulation severely inhibited by appropriate concentration of DFMO. Using the bean rust fungus (Uromyces phaseoli L.) as a test organism, we were able to show that the number of local lesions formed on the first, simple leaves of inoculated bean seedlings was progressively decreased as the concentration of sprayed DFMO was increased. Some protection was conferred by 10 \( \mu M \) half protection by 50 \( \mu M \), and full protection by 500 \( \mu M \) DFMO sprayed onto the plant either 24 h before or after spore inoculation. There was some translocation of the protective effect, since when only half a leaf was painted with DFMO, the entire leaf was at least partially protected; protection was also conferred upon compound leaves that were present only as buds at the time of the DFMO spray. To achieve such protection, DFMO must be applied within about three days of infection, before the fungus has sunk its feeding haustorium into the host cell. Thus, it appears that PA levels in the infected plant can have a profound effect, not only on morphogenesis, but on survival.

DFMO and DFMA have also been used to inhibit PA biosynthesis in higher plants, with consequences for growth. In young tomato fruits, application of DFMO inhibits the cell division necessary for later growth by cell expansion, and such inhibition is reversible by PA application (5). A similar situation pertains in tobacco ovaries, with the additional complication that DFMA is also effective as an inhibitor. This is not due to inhibition of ADC, but rather to an arginine-mediated conversion of DFMA to DFMO, followed by inhibition of ODC. In tobacco ovaries, as in other higher plant systems investigated, ODC and ADC seem to exist in separate compartments of the cell, and the Put to which they give rise may not be freely available to all compartments. Present evidence suggests that ODC is localized to DNA-containing organelles, while ADC is cytosolic.

Malmberg and colleagues (reviewed in ref. 3) have produced presumptive PA mutants in tobacco by regenerating plants from mesophyll protoplasts that survived exposure to high levels of PA-biosynthetic inhibitors. Some of these mutants exhibited aberrant flowering behavior, including altered patterns of floral organ morphogenesis, such as deletion, adnation, replacement and sexual inversion. Since stamens and carpels are the spor-producing organs of the angiosperms, and since PA deprivation had been shown to affect patterns of sporulation in fungi, we decided to investigate the effects of PA deprivation and supplementation on patterns of flowering in tobacco, using the thin layer tissue culture technique (14). Cultures programmed to produce flowers were progressively converted to the vegetative state as the Spd titer was decreased by increasing concentrations of cyclohexylamine, an inhibitor of Spd synthase. Contrariwise, cultures programmed to produce only vegetative buds were induced to produce some flowers upon the addition of 0.5 to 5.0 mM Spd. The effect of cyclohexylamine showed a good dose-response curve and was reversible by applied Spd, but the addition of increasingly higher concentrations of Spd to vegetative cultures did not produce stepwise augmentation of the flowering response. While Spd is apparently specific for inducing such flowering in Wisconsin-38 tobacco, Flores and co-workers found that spermine is most effective with the variety Xanthi.

The interpretation of effects on flowering in these photo-periodically indeterminate tobacco tissue cultures is complicated by the fact that such diverse materials as oligosaccharins and ethylene also induce floral formation. Oligosaccharins may trigger ethylene production, and PAs might work by suppressing or antagonizing ethylene biosynthesis or action. We therefore undertook an examination of the relation between photoperiodic induction and PA titer in a well-characterized short day plant, Xanthium strumarium L. Present evidence indicates that exposure to one to two consecutive inductive long nights greatly increases the titer of conjugates of the higher PAs in leaves, and later in buds (13). This investigation is being extended by a collaborative examination, with Georges Bernier, of the PA content of vascular sap gathered from vegetative controls and induced plants. It is noteworthy that in tobacco, Martin-Tanguy (see ref. 12) has shown an elevated titer of conjugated PAs in flowering, as contrasted with vegetative plants, and especially in flower parts. We have also found Spd binding to a specific protein in tobacco tissue cultures showing a morphogenetic response to Spd (2).

Embryogenesis in tissue cultures appears to depend on high Spd titer, especially in carrot (reviewed by Feirer et al. in ref. 12). Just prior to embryoid formation, such cultures develop high ADC activity and an elevated Spd titer. If DFMA is applied to the cultures, embryogenesis is inhibited, and the inhibition is reversible by applied Spd. It appears possible to adjust the concentration of DFMA so that embryoid formation is inhibited while growth rate is unaffected. In a nonembryogenic line of carrot, the rise in ADC activity and Spd titer do not occur. These facts again suggest a special morphogenetic role for the higher PAs. Similar effects have been noted in protoplast-derived cultures of Vigna and other plants.

POSSIBLE SPECIAL ROLES OF UNUSUAL PAs

In addition to Put, Spd, and Spm, with which we have been concerned, there are also many unusual PAs in nature, which may have special roles. For example, bacteria residing in hot springs contain unusual PAs such as thermospermine, with five or more amino groups, which seem to protect enzymes against heat denaturation (Oshima in ref. 3). Such PAs also complex with and greatly alter the configuration of nucleic acids. In this connection, it is noteworthy that the in vitro transition from the transcriptionally active B-DNA configuration to the inactive Z-configuration is facilitated by attachment of PAs to the DNA, especially at G and C residues (16).

Rapidly growing root nodule bacteria of the genus Rhizobium produce large quantities of aminobutylhomospermidine, a tetraamine not found in slowly growing strains (10). While this compound has also been isolated from Japanese volcanic ash soils, its physiological effects on roots and other plant parts have not been studied.

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LITERATURE CITED