INTERACTION OF GIBBERELLIC ACID & ALLYL TRIMETHYLAMMONIUM BROMIDE UPON GROWTH OF ULOTHRIX 1,2

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The ability of a series of choline analogs to produce a form of dwarfism in wheat was recently described by Tolbert (7). One of the more potent compounds tested was allyl trimethylammonium bromide (AMAB). The most characteristic effect upon the growth of Thatcher wheat after treatment of the roots with AMAB was the development of stockier plants with short thick stems. This pronounced dwarfism is associated with shorter internodal distances. These symptoms appeared quite the opposite of those produced by the application of gibberellin. Therefore, in extending their original observations, Tolbert (8) and Wittwer and Tolbert (9), particularly studied the antagonistic effect of gibberellin on AMAB in both wheat and tomato plants. At sufficiently high concentrations gibberellin was able to reverse completely the morphological effects of the treatment with AMAB. A speculative mechanism was proposed in which the rather high degree of molecular structural specificity observed for growth inhibition activity was compared with the well characterized structural requirements for inhibition of acetylcholinesterase in mammalian systems.

To gain insight into the mechanism of interaction of AMAB and gibberellins we studied their effects upon the growth of the unicellular filamentous green alga, Ulothrix subtilissima. This organism exhibits a particularly large and sensitive response to both indole acetic acid (IAA) and gibberellic acid (GA). At optimal concentrations of IAA a 13-fold stimulation of growth is obtained, both on the basis of net increase of wet weight and increase of cell number as demonstrated by Conrad et al. (2). Some of the advantages and disadvantages in using the algae to study plant growth substances have been reviewed recently by Conrad and Saltman (3). By working with this comparatively simple and undifferentiated system we have been able to demonstrate that not only is AMAB a growth inhibitor at high concentrations, but also is a growth promoting substance at low concentrations. Further, AMAB and GA are competitive inhibitors of each other in a fashion which we are not able to analyze by classical inhibition kinetics.

MATERIALS & METHODS

The procedures followed in this work are identical with those described in our previous paper. Conrad et al. (2). Samples (10 ml) of a modified Bristol's medium used by Bold (1) containing the designated quantities of GA and AMAB were pipetted into screw cap culture tubes and autoclaved for 15 minutes at 121 C. The stability of GA during autoclaving has been demonstrated both in this laboratory and that of Henderson (6). No loss in biological activity of GA is observed when the compound sterilized by heating in the Bristol medium is compared with material sterilized by filtration. A weighed inoculum of Ulothrix subtilissima Rabenh (obtained from the No. 462 Culture Collection of Algae, Indiana University) was introduced into each tube and was incubated at room temperature for 15 days. Natural sunlight augmented during the daylight hours with fluorescent light was the only illumination supplied. For every experiment two tubes for each experimental condition were run. Further, for any given experiment, all concentration variations were carried out at the same time and under the identical environment. By such a technique internal standardization is achieved and any experimental artifact which might arise by variation in growth due to changes in light intensity, temperature, or CO2 concentration is avoided. At the conclusion of incubation, the algae were collected and washed on a tared Millipore membrane filter. The filter and algae were then air dried and reweighed. The values reported are the average weights at each condition. The net increase in weight is directly proportional to increased cell division. Algae from each experimental condition were examined microscopically in a wet mount under magnification of 1,250 X.

RESULTS & DISCUSSION

The response of Ulothrix to increasing concentrations of AMAB is presented in figure 1. There

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was no observable change in the morphology of the cells at any of the concentrations except at lethal levels. It is apparent from these results that AMAB is a growth promoting substance at concentrations of $10^{-6}$ M and lower, but manifests an inhibitory response at higher concentrations. Concentrations of $10^{-2}$ M and higher can cause complete cell destruction. The shape of the growth curve illustrated is typical of those observed for both IAA and GA.

The effect of varying the concentrations of AMAB upon the growth of the algae in a medium containing the optimal concentration of GA is shown in figure 2. It was previously found by Conrad et al. (2) that 50 µg/liter of GA produces the optimal growth response in this alga. It is quite evident that AMAB significantly antagonizes the growth stimulating effect of GA, even when applied in very low concentrations. Thus AMAB, although a growth promoting substance itself, can also be an inhibitor of GA. The general characteristics of this system are similar to those investigated by Foster, McRae, and Bonner (5) in their studies of the interaction of 2,4-dichlorophenoxyacetic acid and IAA in the Avena section test. With this in mind, the next series of experiments was designed to employ the methods of enzyme kinetics to elucidate the nature of the interaction of AMAB and GA. If we were able to determine whether AMAB is a competitive inhibitor or a non-competitive inhibitor, it might be possible to understand more clearly the mechanism of the hormone action.

Algae were grown in sub-optimal concentrations of AMAB in the presence of increasing concentrations of GA, and the net growth was measured (fig. 3). As expected, a definite inhibition of growth was produced. However, all attempts to interpret this data by known equations governing enzyme inhibition kinetics described by Dixon and Webb (4) failed to

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**Fig. 1.** Effect of increasing concentrations of AMAB on the growth of Ulothrix.

**Fig. 2.** The inhibition of growth by increasing AMAB concentrations in a medium containing the optimum concentration of GA, 50 µg/liter.

**Fig. 3.** The interaction of suboptimal concentrations of AMAB with increasing concentrations of GA.

**Fig. 4.** The interaction of optimal and superoptimal concentrations of AMAB with increasing concentrations of GA.
yield any typical linear relationship. Although it is clear that the system is not a non-competitive one, it is not possible to directly state that a simple competitive relationship holds. Calculations were made assuming both a one and one-half and a second order reaction for each of the molecular species, but the non-linear relationship remained.

When growth was studied in culture media containing AMAB at concentrations greater than optimal, $10^{-6}$ M, in the presence of increasing concentrations of GA, similar competition was observed. As shown in figure 4, low concentrations of GA appear to inhibit the growth promoting effect of AMAB. As the concentration of GA increases the situation is reversed and the AMAB begins to inhibit the growth stimulating effect of the GA.

An extensive series of experiments was carried out to determine if there were any interaction of IAA and GA in the Ulothrix system. Over the concentration range of $10^{-8}$ M to $10^{-2}$ M for each of the growth substances no antagonistic or synergistic effect could be observed. When IAA and GA were added together at their respective optimal concentrations, growth was the same as that observed with IAA alone.

It is quite evident that the inhibition of GA activity by AMAB is partially reversible. Adding excess GA to the medium overcomes the depressive effect of AMAB, even when the latter is present in relatively large concentrations. These observations confirm the conclusions of Tolbert (8) and Wittwer and Tolbert (9) that a competitive interaction does exist. Whether this system is one in which the inhibitor combines directly with the growth substance binding site, or a partially competitive system in which the inhibitor affects the affinity of the enzyme for substrate remains to be clarified. It is difficult to envision any structural similarity between GA and AMAB which would support the analogy between this system and that for cholinesterase inhibition. At present we are not able to offer a definitive mechanism for the action of AMAB in the algal system. Further investigations are in progress to test a number of possible hypotheses to clarify the biochemical events which lead to the stimulation and inhibition of growth of algae and higher plants by GA and AMAB.

**Summary**

I. The interaction of allyltrimethylammonium bromide (AMAB) and gibberellic acid (GA) on the growth of *Ulothrix subtilissima* has been studied.

II. AMAB can function both as a growth substance at low concentrations and a growth inhibitor when applied at high concentrations.

III. Experiments are presented to demonstrate that there is a mutual competitive inhibition of AMAB and GA. However, definitive interpretations of these results by means of enzyme inhibition kinetics are not possible.

IV. No morphological changes due to the application of AMAB or GA were observed.

**References**