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Effects of Inorganic Solutes on the Binding of Auxin[1]

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ABSTRACT

The binding of α-naphthaleneacetic acid (^{14}C-NAA) to pelletable particulates from corn (*Zea mays*) coleoptiles was found to be influenced by inorganic solutes. La^{3+}, Ca^{2+}, and Mg^{2+} increased the binding whereas monovalent cations did not. The concentrations of $CaCl_2$ which increased auxin binding were similar to those which inhibited coleoptile elongation in the presence of auxin. These results are interpreted as suggesting that the alteration of hormonal effectiveness by some inorganic solutes involves alterations in the attachment of the hormone to binding sites in the cell.

Hormonal regulation at the cellular level is generally considered to involve binding of the hormone to a membrane or other intracellular surface, both for plant (19) and animal (13) hormones; it is also known that the fine structure of membranes and macromolecules can be altered by inorganic solutes through influences on surface charges and on water lattices (18). It would seem an attractive possibility, then, that inorganic solutes might represent a previously unappreciated factor in the function of plant hormones. Having found that regulatory functions of each of the plant hormones are susceptible to alteration by inorganic solutes (4, 7, 8), it becomes of special interest to determine whether such solutes can also alter the attachment of hormones to membranes from hormone-responsive tissues.

The most notable case of a plant hormone being experimentally attached to a cell membrane is the specific binding of auxin to a particulate fraction from corn coleoptiles (2, 15, 16). The present experiments describe some effects of inorganic salts on the specific binding of the auxin, NAA,[4] to such particulates.

MATERIALS AND METHODS

Corn seedlings (*Zea mays* L., Gro-Mor seed corn from Harger Farms, Noblesville, Ind.) were grown in the dark with 1 hr of red light/day for 7 days, as described by Hertel and Flory (1).

Growth tests were carried out with 8-mm sections of coleoptiles in solutions of various concentrations of salts, with or without 10^{-5} M IAA; the final length was read after 16 hr.

Particulate fractions from coleoptiles were prepared following the method of Thomson and Leopold (15). Typically, 40 g of whole coleoptiles were ground in a cold mortar and pestle with 100 ml of grinding medium and a little acid-washed sand. The medium contained 250 mM sucrose, 40 mM tris buffer, and 1 mM EDTA at pH 8.3. The liquid was squeezed through one layer of Miracloth with two layers of cheesecloth on the outside. The crude homogenate was centrifuged at 2,000*g* for 10 min to remove cell walls. The supernatant was centrifuged again at 30,000*g* for 20 or 40 min and the pellet was suspended in 36 ml of the binding medium at pH 5.5 (250 mM sucrose, 10 mM HEPES buffer with the pH adjusted to 5.5 with tris). All operations were done at 0 to 4 C.

Desired concentrations of cations were added to an aliquot of sample along with radioactive NAA (50 mCi/mmol) at 5×10^{-7}M. To another aliquot of homogenate were added both radioactive NAA at 5×10^{-7} M and an excess of nonradioactive NAA at 10^{-4} M. One ml of a sample in triplicate from each treatment was spun in cellulose acetate-butyrate tubes as described earlier (2, 15) in an SS 34 rotor at 30,000*g* for 20 or 40 min. The supernatant was decanted and the pellet was cut out with the bottom of the tube. The entire pellet and 0.1 ml of the supernatant were transferred separately to scintillation vials containing Bray's solution. The radioactivity was measured in a Beckman scintillation counter. Specific binding was calculated as the percentage difference in radioactivity in a pellet from a sample containing ^{14}C-NAA (5×10^{-7} M) alone and from a sample containing both ^{14}C-NAA (5×10^{-7} M) and unlabeled NAA (10^{-4} M). The amount of radioactivity from the auxin which was displaced by the excess unlabeled auxin is taken as the amount of specifically bound auxin (2). All of the binding experiments were repeated on at least six different occasions. For protein determination, the pellet was dissolved in 0.1 M NaOH and an aliquot was used for analysis by the method of Lowry *et al.* (5).

RESULTS

To examine the effects of some inorganic solutes on growth responses of corn coleoptiles to auxin, 8 mm sections of coleoptiles were placed in solutions of various concentrations of $CaCl_2$ and $(NH_4)_2SO_4$, in the absence or in the presence of 10^{-5} M IAA. These salts were selected as representatives of the two extremes of the Hofmeister series, $CaCl_2$ being a strong destabilizer and $(NH_4)_2SO_4$ being a strong stabilizer in the series. Figure 1 shows that in the absence of the auxin, the salts had little effect on elongation, but in the presence of the auxin, $CaCl_2$ had a marked inhibitory effect, and $(NH_4)_2So_4$ had a lesser promotive effect. A 50% inhibition of the auxin stimulation effect was obtained at about 10^{-3} M $CaCl_2$.

In order to examine the effects of calcium concentrations (0.01-10 mM) on auxin binding, a comparison of radioactivities in pellets from the ^{14}C-NAA (5×10^{-7} M) samples and from samples containing ^{14}C-NAA plus unlabeled NAA (10^{-4} M) in the presence of various concentrations of $CaCl_2$ are shown in Figure 2. The results show that the higher concentrations of $CaCl_2$ increased the amount of radioactivity in the pellets. In order to be sure that these increases were not reflections of changes in pellet size, protein contents of the pellets were measured, and the data did not indicate any significant change in the

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[4] Abbreviation: NAA: naphthaleneacetic acid.

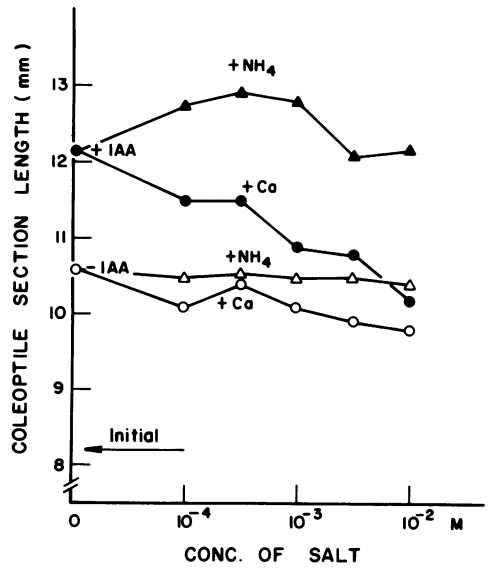

FIG. 1. Effects of $CaCl_2$ and $(NH_4)_2SO_4$ on the growth of corn coleoptile sections in the presence and absence of the auxin, IAA (10^{-5} M).

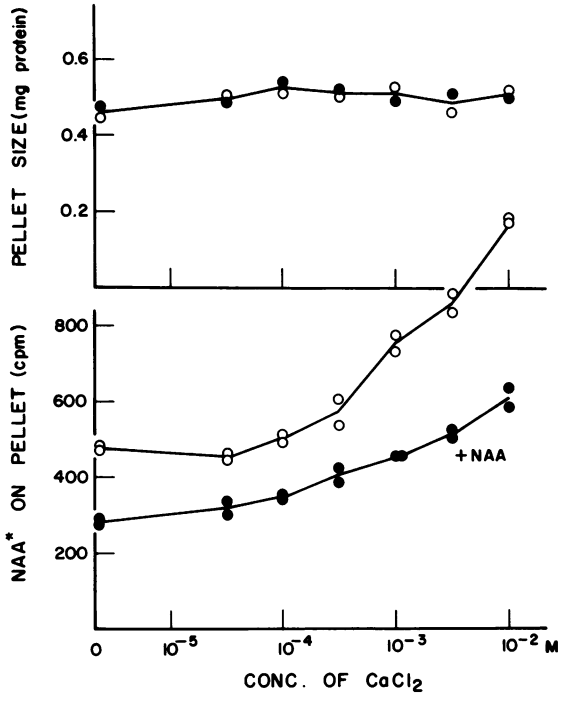

FIG. 2. Effects of calcium on the radioactivity in pellets of corn coleoptile membranes from ^{14}C-NAA (5×10^{-7} M) (○) and from ^{14}C-NAA (5×10^{-7} M) plus unlabeled NAA (10^{-4} M) (●). Upper plot shows the pellet size at various calcium concentrations.

presence of $CaCl_2$ as compared to the control (Fig. 2, upper curve). The specific binding of NAA at various calcium concentrations is shown in Figure 3. At the three highest concentrations tested, Ca increased the specific binding, causing about a 50% increase in binding at about 10^{-3} M $CaCl_2$. At lower concentrations, the binding was slightly lower than that of the control.

Effects of different cations on auxin binding are shown in Table I. Chlorides of La^{3+}, Ca^{2+}, and Mg^{2+} each increased the binding, whereas K^+, Na^+, and NH_4^+ did not. Lanthanum greatly increased auxin binding and NH_4^+ slightly inhibited binding. Measurements of pellet size show that the increases in binding by divalent and trivalent cations were not due to increases in pellet size. The relative effectiveness of the tested cations in increasing auxin binding is in the following order: La > Ca > Mg > K, Na > NH_4.

DISCUSSION

It has been known for many years that inorganic solutes could have profound effects on growth, especially in the presence of auxin (14). The experiments reported here have shown that in addition to altering growth, inorganic solutes can strongly modify the binding of auxin to the pelletable particles from corn coleoptiles. The effects of $CaCl_2$ enhancing auxin binding occur at roughly the same concentrations as the effects inhibiting growth.

The density of binding sites can be estimated from the specific binding data; with a counting efficiency of 60%, the 200 cpm of NAA specifically bound indicate that there may be approximately 6.6×10^{-11} mol bound/mg N in the pellet without added $CaCl_2$. In the presence of 10^{-3} M $CaCl_2$, the binding was increased to 300 cpm, which indicates approximately 10^{-10} mol bound/mg N. These values are slightly higher than the values reported by Hertel *et al.* (2).

Any attempt to explain the growth inhibition effect of $CaCl_2$ by the observed enhancement of auxin binding will be confounded by two major uncertainties: (*a*) it is uncertain whether the auxin-binding site on pelleted membranes is the site at which the regulation of growth is achieved; it is possible that this binding site is more specifically related to auxin transport (2); and (*b*) it is uncertain whether an increased binding of auxin to its site of action would be expected to increase the growth

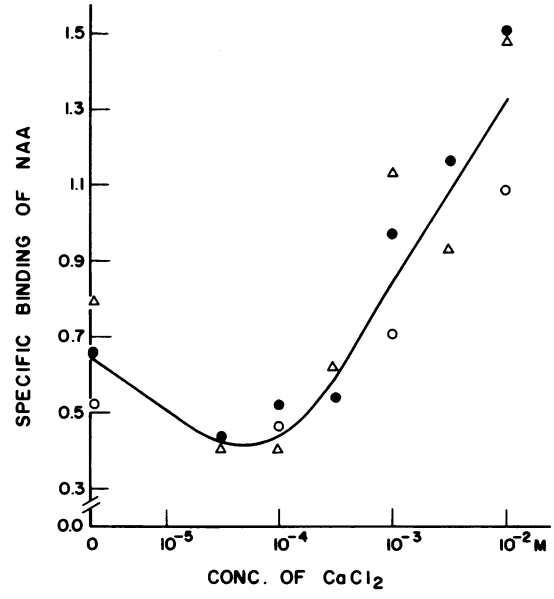

FIG. 3. Effects of various concentrations of $CaCl_2$ on the specific binding of NAA to corn coleoptile membranes.

Table I. Effects of Various Cations on NAA Binding to Particulate Fractions of Corn Coleoptiles and Their Effects on Pellet Size

Treatment (10 mM salt)	Specific Binding of NAA	Pellet Size
		mg protein
Control	0.522	0.54
+$LaCl_3$	3.696	0.46
+$CaCl_2$	1.125	0.42
+$MgCl_2$	0.800	0.48
+KCl	0.489	0.62
+NaCl	0.448	0.58
+NH_4Cl	0.380	0.64

response or to decrease it. Although any explanation of the $CaCl_2$ effects on growth as an effect on auxin binding must remain only a possibility, it is nevertheless clear from these experiments that salts such as $CaCl_2$ can alter the binding of auxin to specific binding sites, and this opens the possibility of inorganic salts altering the hormonal functions of auxin through alterations of its binding sites in the cell.

Hofmeister (3) notes that inorganic solutes had a range of effects on the solubilities of macromolecules, ranging from extreme solubilizing effects by Ca salts to insolubilizing effects by NH_4 salts. The Hofmeister series is more currently described as destabilizing and stabilizing effects of solutes on macromolecules (18). The order of effectiveness of solutes compared in the binding experiments is not dissimilar to the Hofmeister series, or the destabilization/stabilization series. The notable effectiveness of lanthanum as a destabilizing agent, or "supercalcium," is consistent again with the solute effects described by von Hippel and Schleich (18), and its profoundly effective inhibition of coleoptile growth was described by Pickard (6). The fact that Ca and NH_4 salts show strikingly different effects on hormonally regulated functions in plants (4) offers a suggestive parallel to the effects of these salts on membranes and macromolecules (9); the present experiments might be interpreted as indicating that the solute effects on hormonal functions are related to the effects of the solutes on hormonal binding sites. A distinctive role for Ca in animal hormone functions has already been described (10–12).

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