Planta (Berl.) 91, 314—320 (1970)

Comparison of Calcium and Lanthanon Ions in the *Avena*-Coleoptile Growth Test

Barbara G. Pickard
Center for the Biology of Natural Systems and Department of Biology, Washington University, Saint Louis, Missouri

Received December 20, 1969

Summary. Inhibition of elongation of oat coleoptile sections by La^{3+}, Pr^{3+}, and Nd^{3+} is similar to but greater than that by Ca^{2+} at low and intermediate concentrations. The lanthanons might serve as probes to learn more about the manner of action of Ca^{2+}, a normal growth-regulating agent.

Introduction

The control of coleoptile elongation by calcium ions has long been of interest to plant physiologists, for it has been hoped that study of this naturally occurring growth inhibitor might lead to a deeper understanding of the process of growth itself (Burling and Jackson, 1965). Since one of the most conspicuous properties of the Ca^{2+} ion is its divalence, hypotheses about its mode of inhibition have often focused on the role of the two net positive charges. However, both the specific role of the double charge and the general mechanism of interference by the ion remain unknown. The present study undertakes a preliminary comparison of the inhibition due to Ca^{2+} ions with inhibitions by three of the lighter lanthanons, in order to see if indeed these ions might profitably be used in further studies on control of elongation.

The chemistry of the lighter lanthanons resembles that of calcium in many respects, but their ions differ in having higher atomic weights and an additional positive charge. Therefore, substitution of a lanthanon for a Ca^{2+} ion might influence a biochemical reaction in one of four ways: (1) lanthanons might mimic Ca^{2+} closely, suggesting that the charge requirements for the interaction are already saturated by the double positive charge of Ca^{2+}; (2) action by lanthanons might be much stronger than that by Ca^{2+}, suggesting that interactions of Ca^{2+} are limited by its divalence; (3) lanthanons might have no influence on a reaction in which Ca^{2+} participates, suggesting that the other reactants have a strong specificity for Ca^{2+}; (4) a lanthanon might act in opposition to Ca^{2+}, binding competitively or noncompetitively but failing to carry out further action. Most commonly, a lanthanon might be expected to replace Ca^{2+} with equal or greater effectiveness. Comparative biological studies are rare (Steidle, 1935; Trombe *et al.*, 1959); however, Takata *et al.* (1966) have referred to lanthanum itself as "supercalcium" in describing its effect

on ionic conductances in the lobster axon membrane. Thus, experiments with lanthanons might show to what extent the participation of Ca^{2+} in one or more growth reactions is limited by ionic charge. Perhaps more important, however, is the possibility of establishing a characteristic of the Ca^{2+} inhibition which might be used to identify the susceptible reaction system during future attempts to locate it in the cell and to isolate it in the test tube: Ca^{2+} takes part in many biological reactions, but the effect of substituting lanthanum for Ca^{2+} may differ from system to system. One reasonable criterion for the involvement of a specific Ca^{2+}-influenced reaction in the limitation of elongation could be similar *in-vivo* and *in-vitro* responses to the lanthanon ions.

Interest in comparing the growth-limiting behavior of more than one lanthanon ion rises from the slight differences between the members of the series. Lanthanum, praseodymium and neodymium have long been difficult to separate from each other by ordinary chemical means, but use of ion exchange resins now permits clean separation. The electronic behavior of praseodymium is more complex than that of lanthanum, as the former can exist either as a tri- or tetravalent ion. The crystal radii of La^{3+}, Pr^{3+}, and Nd^{3+} decrease in the listed order. Thus, comparison of growth inhibition by these three ions might provide finer distinguishing characteristics to aid in the search for the particular chemical reaction or reactions inhibited by Ca^{2+} than would study of a single lanthanon. For documentation of these and other properties of the lanthanons, see Moeller (1963) and Vickery (1961).

Materials and Methods

$LaCl_3$, $PrCl_3$, and $NdCl_3$ were prepared by combining a quantity of the lanthanon oxide with slightly more than 6 times the number of moles of HCl. Unreacted HCl was evaporated under reduced pressure. The 99.99% pure La_2O_3 was obtained from Matheson, Coleman and Bell, Norwood, Ohio, and the 99.9% pure Pr_2O_3 and Nd_2O_3 were from A. D. Mackay, Inc., New York, N. Y.

Initial experiments were carried out on both wheat and oat coleoptiles; the response patterns were similar but the wheat was much more variable. The experiments of Figs. 1 and 2 were therefore performed on *Avena sativa* L. cv. Victory (USDA CI 2020), supplied by the U. S. Department of Agriculture Branch Station at Aberdeen, Idaho. Seedlings were grown under conditions described elsewhere (Pickard *et al.*, 1969), but with roots in deionized water rather than in agar. 10 mm sections were cut with a double-bladed instrument, discarding the apical 2 or 3 mm of the coleoptile and removing the primary leaf, and sets of 10 were floated in 10 ml of solution in a 9 cm Petri dish. All solutions contained 5×10^{-2} M sucrose, 10^{-4} M potassium salt of penicillin G, and, except for the control without auxin, 2×10^{-5} M 3-indoleacetic acid; other additives are specified in the figures. 12 hr after the beginning of the experiment, all sections were placed in fresh solutions.

Results

As shown in Fig. 1, $LaCl_3$ was slightly more inhibitory than was $CaCl_2$ at concentrations of 1 mM or less but considerably less inhibitory at 3 mM or more. Sections treated with the highest concentrations of either salt became very crisp and turgid, but this effect was much more pronounced for $LaCl_3$ than for $CaCl_2$ treatments. Fig. 2 shows that the time course of elongation in the presence of $LaCl_3$ was not markedly

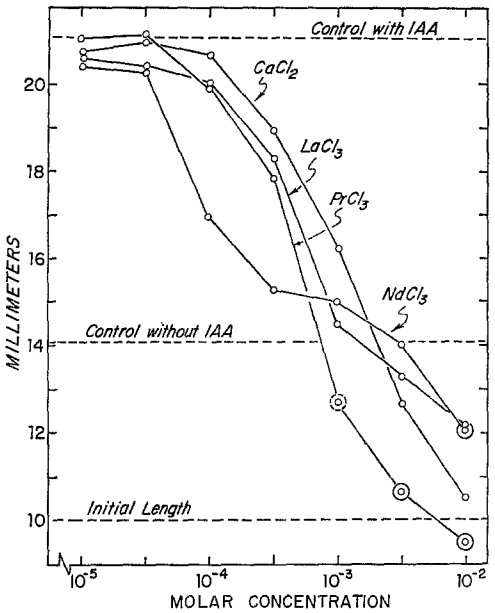

Fig. 1. Influence of $CaCl_2$, $LaCl_3$, $PrCl_3$ and $NdCl_3$ on elongation of *Avena* coleoptile sections in the presence of IAA. Length was measured at 24 hr. Data points are averaged from 4 replicate experiments; elongation of 40 sections is averaged for the highest 4 concentrations and of up to 120 sections for the lower concentrations and for the auxin control. Circled points represent sections with little or no turgor

different from that in the absence of added polyvalent ions, except that it was much slower and the final length attained at the end of a day was lower.

Praseodymium did not appear to inhibit growth significantly below 10^{-4} M, but at higher concentrations inhibited even more effectively than did $LaCl_3$ (Fig. 1). However, at high concentrations, the inhibition by $PrCl_3$ is dissimilar to that by $LaCl_3$: sections floated in 10^{-3} M $PrCl_3$ were slightly limp and sections floated in still higher concentrations were yellowish and very flaccid. In fact, sections floated at 10^{-2} M shrank to about 95% of their initial length.

Fig. 2 further shows that sections floated on 3 mM $PrCl_3$ grew at an initial rate only slightly lower than controls, but that by 4 hr the rate fell off and that actual shrinkage occured within 6 hr. The decrease in growth rate at 4 hr was accompanied by a slightly flaccid appearance of the ends of each section; the flaccid region never extended more than 1 mm away from the cut surface at this time. By 8 hr the sections were limp along their entire length, and by 24 hr the sections were so flaccid that when suspended at the midpoint over a needle, their halves drooped vertically downward. In sum, the initial effect of $PrCl_3$ seemed slight, but ultimately, working inward from the cut surfaces, $PrCl_3$, decreased cellular turgidity.

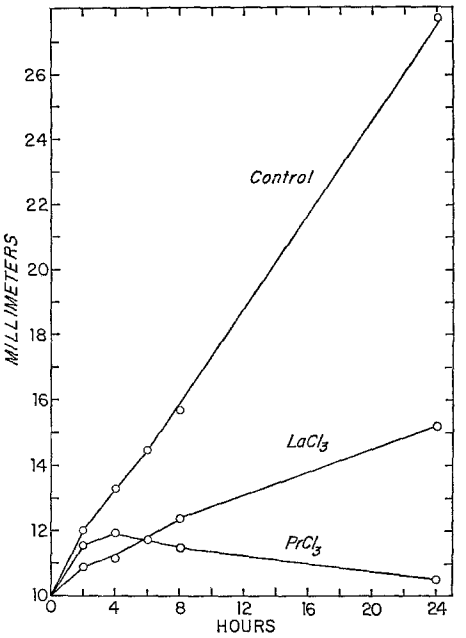

Fig. 2. Section length as a function of time of exposure to control solution, 3 mM $LaCl_3$ and 3 mM $PrCl_3$. IAA was present in treatments and controls. Data points are averaged from 3 experiments involving a total of 4 sets of 10 sections each for the control and a total of 6 such sets for the lanthanons

Fig. 1 shows that $NdCl_3$ is the most effective of the three lanthanon inhibitors at low concentrations. The slope of the inhibition curve levels off, however, only to steepen again at very high concentrations. At 10^{-2} M $NdCl_3$, the sections were flaccid. Thus, although the inhibition of growth by the solution of 10^{-2} M $NdCl_3$ is numerically the same as that by the same concentration of $LaCl_3$, the mechanism of inhibition at this concentration may resemble that of $PrCl_3$.

Discussion

Within a range of low concentrations, added lanthanum is a slightly more effective inhibitor of coleoptile elongation than is added calcium. The close parallelism of the inhibition curves for the two substances at concentrations below 1 mM suggests that the same site of action is involved up to this point. Thus, the charge of Ca^{2+} is probably somewhat suboptimal for its inhibitory action on growth.

The conspicuously greater stiffening of the sections by 10^{-2} M lanthanum than by calcium occurs in spite of the lesser inhibition of growth. A number of explanations might be proposed for the stiffening;

one of the most interesting is that calcium and lanthanum decrease the permeability of the cell membrane to other ions such as potassium without altering the ion pumps, so that water has a greater tendency to enter and remain in the cells. Some credibility is lent to this suggestion by the observation that 10^{-2} M neodymium and praseodymium bring about flaccidity; all three lanthanons might tend to displace Ca^{2+} from the sites where it is bound to the membrane, but once bound, lanthanum might act like "supercalcium" while praseodymium and neodymium might contribute no stiffening action. It might be relevant that the greatest wilting is produced by the ion with the strongest tendency to exist in more than one state of oxidation. Another possible explanation of the stiffening is that the lanthanons, which are known to precipitate nucleic acid and nucleoproteins (Hammarsten and Teorell, 1928) might influence the synthesis of some substance required for membrane integrity or activity. Still another possibility is, of course, direct or indirect action on the cell wall; however, Fig. 1 makes clear that a control of cell wall properties cannot completely explain the extent of inhibition of elongation for high concentrations of the inhibitors.

The failure of praseodymium to inhibit growth at low concentrations found inhibitory for lanthanum may be illusory; the absolute differences measured for the two ions are small. It is also possible that praseodymium ions are more strongly complexed with the glass dish or with substances in the medium than are lanthanum ions, or they may be differentially bound and localized at the surface of the sections or at irrelevant sites within the tissue. Indeed, it must be stressed that at extremely low concentrations the binding of lanthanon ions to glass is likely to be so proportionately strong that the ultimate concentration of ions in solution may be significantly lower than that prevailing at the outset. In the experiment of Fig. 1, however, binding to glass would not be expected to influence data for higher salt concentrations or to influence the basic pattern of results, but only to decrease the apparent sensitivity of the sections to very low applied concentrations of the inhibitors.

The strong inhibition by low concentrations of neodymium, which has a crystal radius slightly smaller than that of lanthanum or trivalent praseodymium and extremely close to that of Ca^{2+}, is particularly interesting in view of the leveling-off of the effect at about 1 mM. It is hard to believe that sites of action are already saturated at this concentration, considering that the curves for lanthanum and calcium drop to greater values of inhibition before inflecting. Also, it is worth noting that calcium, lanthanum, and neodymium stand in the same sequence whether ordered for strength of inhibition at low concentrations, for lowness of concentration at which inflection of the inhibition curve occurs, or for lowness of inhibition at the point of inflection. This way of

thinking about the curves emphasizes a similarity of trend which might speak for participation of the ions in a single reaction system. On the other hand, it is just as plausible to postulate that at higher concentrations these three ions take part in a second reaction which opposes the growth-inhibiting mechanism. When evaluating response curves of biological systems to heavy metals, it must always be kept in mind that a great variety of reactions may contribute to the ultimate response, and that more than one of these may be susceptible to the influences of the applied inhibitors. Indeed, simultaneous inhibition of more than one growth-controlling reaction could even explain the sharp initial drop of the neodymium curve. Finally, it should be noted that the role of osmotic potential due to high concentrations of the salts was not checked; this factor might possibly contribute to the inhibition of growth by a salt concentration such as 10^{-2} M.

In spite of some intricacies in interpretation of details of the inhibition curves, the apparent similarity of action by calcium and lanthanum encourages further investigations. Since at low and medium concentrations lanthanum appears to be a slightly more effective inhibitor of growth than is calcium, it would seem worthwhile to compare activities of the two ions in any specific reactions suspected of being the Ca^{2+}-governed step in the growth sequence. Initially, the relationship of the inhibition of growth by Ca^{2+} and its analogs to mechanical properties of the cell wall deserves testing by the Instron technique of Cleland (1967). Moreover, since at intermediate concentrations inhibition by praseodymium, neodymium, and lanthanum may prove similar, sensitive optical spectroscopy with the former two ions might permit observations on the kinetics of binding (see, for example, Spedding, 1940; McLaughlin and Conway, 1963; Wong *et al.*, 1963; Crosswhite *et al.*, 1965; Wybourne, 1965).

The possibility that the strong wilting action of praseodymium is correlated with transformation to the tetravalent ion could be checked by means of a spectrophotometer (Asprey, 1961). Moreover, praseodymium, unlike lanthanum and calcium, is paramagnetic, and although its absorption bands are too broad to permit easy detection of changes in the electronic distribution which might result from binding, they would undergo marked changes when an electron shifts out of the valence shell.

If electron spin resonance measurements of binding by Ca^{2+} analogs should seem desirable, the lanthanon gadolinium might be assayed as a growth inhibitor. Gadolinium resembles lanthanum, neodymium, and praseodymium closely, and has an ionic radius only slightly smaller than Ca^{2+} (Gd^{3+} 0.94 Å, Ca^{2+} 0.99 Å, La^{3+} 1.02 Å). Changes of the gadolinium ion associated with binding might reflect both the altered distribution of electrons about the nucleus (Wybourne, 1966) and the lowered tumbling rate of the ion associated with attachment to a protein of high bulk and molecular weight. (Stone *et al.*, 1965).

Yet another possible means of following kinetics of interaction is suggested by the finding of Katzin and Gulyas (1962; see also 1968) that the rotatory dispersion of tartrate (used as a model optically active compound) in aqueous solution is strongly influenced by binding with Ca^{2+} and praseodymium ions. If indeed Ca^{2+} inhibits growth by binding to an optically active portion of a molecule, such a technique might permit monitoring the behavior of Ca^{2+} itself, as well as of its lanthanon analogs.

This study was supported through the Center for the Biology of Natural Systems by National Institutes of Health Grant No. 5 P10 ES00139. I am extremely grateful for the resourceful assistance of Kathleen Dutson, and I wish to thank Dr. J. H. Burgess and Dr. S. I. Weissman for helpful advice.

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Barbara G. Pickard
Biology Department
Washington University
St. Louis, Missouri 63130, U.S.A.