Planta (1986) 167:527–535

Planta

Effect of auxin and abscisic acid on cell wall extensibility in maize coleoptiles

U. Kutschera and P. Schopfer*
Biologisches Institut II der Universität, Schänzlestrasse 1, D-7800 Freiburg, Federal Republic of Germany

Abstract. Plastic and elastic in-vitro extensibilities (E_{pl} and E_{el}) of cell walls from growing maize (*Zea mays* L.) coleoptile segments were measured by stretching frozen-thawed tissue, pre-extended to its in-vivo length, at constant force (creep test) in a custom-buildt extensiometer, equipped with a linear-displacement transducer. The indole-3-acetic acid (IAA)-induced change of E_{pl} (ΔE_{pl}) is strictly correlated with the growth rate for a period of 3–4 h. Subsequently, ΔE_{pl} remains constant while the growth rate is slowing down. Since this discrepancy can be accounted for by a growth-dependent reduction of osmotic pressure, it is concluded that ΔE_{pl} represents quantitatively the relative increase of in-vivo extensibility (cell wall loosening) involved in IAA-mediated cell growth over a much longer time. On the other side it is argued that the growth rate may not be strictly correlated with wall extensibility during long-term growth. Abscisic acid (ABA) inhibits segment growth induced by auxin, fusicoccin, or exogenous acid, and this effect can be quantitatively attributed to an ABA-mediated reduction of cell wall extensibility as determined by the ΔE_{pl} measurement. Both, IAA and ABA have no effect on total protein synthesis, RNA synthesis, and amount of osmotic solutes. Fusicoccin-induced proton excretion is only slightly inhibited by ABA. In contrast to ABA, growth inhibition by cycloheximide (CHI) is always much larger than the concomitant reduction of ΔE_{pl}, indicating that a further growth parameter is also involved in the inhibition of cell growth by CHI. ΔE_{el} is not affected by either IAA, ABA, or CHI. It is concluded that ΔE_{pl}, as determined by the applied method, represents a relative measure of the actual in-vivo extensibility of the growing cell wall at the very moment when the tissue is killed, rather than an average extensibility accumulated over some immediate-past period of time as suggested by Cleland (1984, Planta **160**, 514–520). Hence, we further draw the conclusion that IAA and ABA control of cell growth can entirely be attributed to a modulation of cell wall extensibility by these hormones in maize coleoptiles.

Key words: Abscisic acid – Auxin – Cell wall (extensibility) – Creep (cell wall) – Extensibility (cell wall) – Growth (auxin, abscisic acid) – *Zea* (coleoptile).

Introduction

Auxin (IAA) causes cell elongation in coleoptiles and other plant organs by anisotropically increasing the plastic extensibility (m) of the cell wall, a structure possessing viscoelastic properties (Heyn 1931, 1933). Despite many efforts, however, it is still unclear whether m is the only target of hormonal growth control and if it really determines cell extension also during long-term growth. One reason for this unsatisfactory situation resides in the difficulties inherent in the measurement of cell wall extensibility of growing tissues. Basically, there are three experimental approaches to this problem which have repeatedly been reviewed (Cleland 1971a, 1981; Masuda 1978; Taiz 1984). The most widely applied method makes use of the Instron testing machine, measuring the mechanical stress of isolated walls extended with a constant strain rate (Cleland 1967). Alternatively, some workers prefer the measurement of stress relaxa-

* To whom correspondence should be addressed

Abbreviations: ABA = ± abscisic acid; CHI = cycloheximide; E_{el}, E_{pl} = elastic and plastic in vitro extensibilities, respectively ($E_{el} + E_{pl} = E_{tot}$); FC = fusicoccin; IAA = indole-3-acetic acid

tion kinetics in walls held at a constant strain (Masuda 1978); and finally it is possible to measure the extension rate of walls subject to a constant stress (creep test). Although the latter technique is obviously the one most closely resembling unidirectional cell extension by turgor pressure, it has only very rarely been exploited in the physical analysis of growth (Cleland 1971b; Penny et al. 1974). Even though a systematic comparison is lacking, it is assumed that comparable results could be obtained by means of these three techniques (Cleland 1981; Taiz 1984) which are, however, principally unable to provide direct information on the cell wall extensibility controlling cell extension in vivo. Firstly, wall extensibility is measured, using killed tissue, at extension rates much higher than during normal growth. The applied force produces only a brief deformation (overstretching) response rather than a reflection of the steady-state wall extensibility, continuously used up and reformed in the living tissue. Secondly, these mechanical tests probe extensibility at a unidirectional force whereas turgor pressure exerts a multidirectional force upon the cell walls. Despite these fundamental theoretical differences it is, nevertheless, conceivable that measurements of cell wall extensibility in vitro, via one of these techniques, can provide an empirically valid, relative measure of the physiological parameter m, at the very moment when the tissue is killed. Unfortunately, this expectation has so far only been verified in qualitative terms and has even led to contradictory results in some instances (see Taiz 1984 for a review). Kinetic discrepancies between growth rate and plastic wall extensibility, measured with isolated walls in the Instron under various experimental conditions, led Cleland (1984) to the conclusion that this technique does not measure actual (relative) values of m; but average values of m over some immediate-past period of time. This view implies, for instance, that, in addition to wall loosening events that are converted into extension, IAA produces also wall-loosening events which are not transformed into extension by turgor pressure in vivo, but can be detected by the Instron. On the contrary, Masuda (1978) proposed from his experiments with *Avena* coleoptiles that in-vitro extensibility represents the potential for future cell extension.

We have been confronted with this problem in our current efforts to elucidate the action mechanism of abscisic acid (ABA) in cell growth. Recent investigations with germinating seeds have shown that ABA inhibits cell expansion – and thereby germination – of the embryo by interfering with cell wall loosening (Schopfer and Plachy 1985). In view of the fact that germinating seeds are not appropriate for a direct physical analysis of cell wall extensibility, it was of interest to investigate the action of ABA in a more convenient system, such as the grass coleoptile, in which IAA-mediated elongation can likewise be rapidly inhibited by ABA (Zenk 1970; Rehm and Cline 1973a; Philipson et al. 1973). There is evidence that ABA reduces the extensibility of cell walls in leaves (Deschamp and Cooke 1984; Van Volkenburgh and Davies 1983).

Thus, the aim of the present investigation was twofold. As a first step we tried to find an answer to the question of whether or not in-vitro extensibility measurements by the creep test are in fact of no use for a quantitative assessment of m during hormone-controlled growth. After finding a satisfactory correlation between growth rate and plastic cell wall extensibility, as measured with this technique in the IAA-induced growth response, we secondly analyzed the effect of ABA on the in-vitro extensibility of IAA-treated tissue. The action of the protein-synthesis inhibitor cycloheximide (CHI) was also included in this study for comparison.

Material and methods

Preparation of intact or abraded, subapical segments (10 mm long, 3 mm below the tip) of etiolated maize (*Zea mays* L. cv. Brio) coleoptiles and the methods for measuring extension growth and simultaneous proton excretion (in 0.1 mmol·l^{-1} $Ca(OH)_2$, 25° C) in the presence of growth regulators were previously decribed in Kutschera and Schopfer (1985a: Fig. 1a, b). The ±ABA was obtained from Fluka (Buchs, Switzerland), and fusicoccin (FC) was a gift from Dr. B. Lercari (Pisa, Italy). All experimental treatments were performed under normal laboratory light at 25° C. In-vitro extensibility was measured at 25° C in segments taken from the growth recorder at various times, trimmed to 10 mm, and fixed with cyanacrylate glue between the lower and upper needles of the constant-load extensiometer, as shown in Fig. 1. The routine procedure for measuring total, elastic (reversible) and plastic (irreversible) extensibilities (E_{tot}, E_{el}, E_{pl}) is outlined in Fig. 2 using the recorder tracings of a typical experiment. Osmolality of expressed cell sap was determined cryoscopically using a Vogel (Giessen, FRG) osmometer 0M 801. For the determination of leucine and uridine incorporation into total protein and RNA, respectively, 10 non-abraded segments were incubated at 25° C on a shaker in scintillation vials containing 1 ml solution with either 3.8 kBq L-[4,5-^{3}H] leucine (1.85 TBq·mmol^{-1}) or 3.8 kBq [5-^{3}H]uridine (1.1 TBq·mmol^{-1}) and IAA (ABA), as specified in the legend of Fig. 7. After 1–4 h the samples were extracted with trichloracetic acid (containing unlabeled tracer), methanol-formic acid, and ethanol-ether according to Gulati et al. (1979). The tissue was suspended in 500 µl Soluene-350 (Packard, Frankfurt, FRG) and the radioactivity counted using a toluene-based scintillation cocktail.

All data points are means (±estimated standard errors) of 4–12 independent experiments. In the growth measurements standard errors were below 2%.

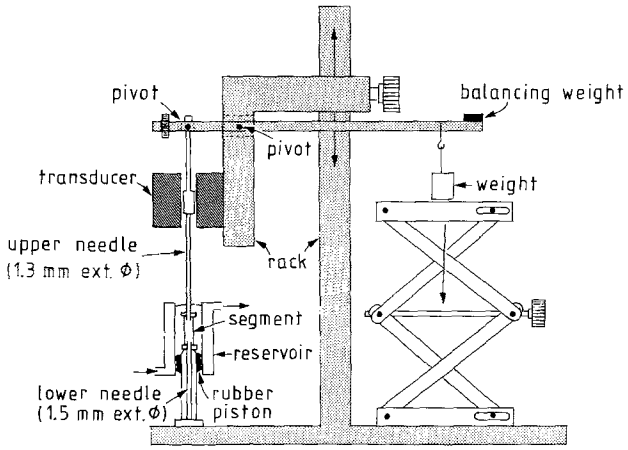

Fig. 1. Apparatus for measuring the in-vitro extensibility of maize coleoptile segments using a linear-displacement transducer (Serie 605-12,7; Erichsen, Wuppertal, FRG; sensitivity 0.0165 mV·μm^{-1}). A segment is fixed between the ends of two hollow stainless-steel needles (equipped with tightly fitting 1-mm pieces of tygon tubing) by means of cyanacrylate glue. The medium reservoir can be moved vertically on a rubber piston. The upper extension of the transducer core is attached to the short arm of a balanced asymmetric lever (1:4). The long arm of the lever can be loaded with a weight by lowering the jack

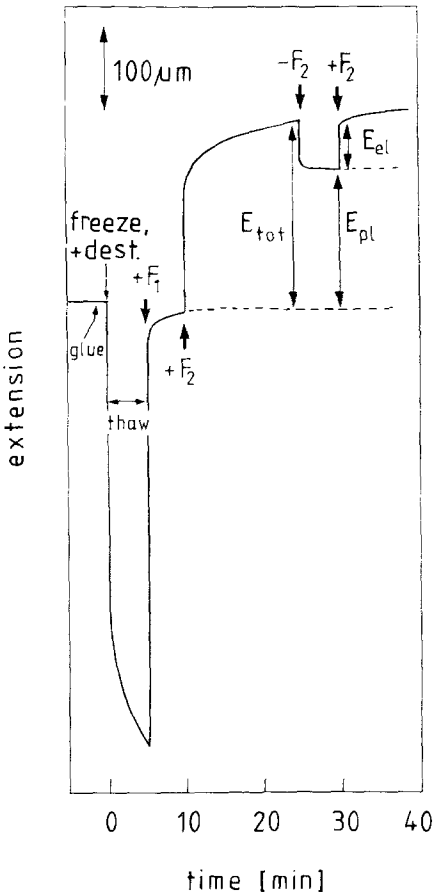

Fig. 2. Chart-recorder tracing (12 cm·h^{-1}) showing the changes in length of a coleoptile segment during the measurement of in-vitro extensibility in the apparatus shown in **Fig. 1** (non-abraded segment treated for 6 h with 6 μmol·l^{-1} IAA). After allowing the glue to set for 1 min, the segment was frozen by spraying it briefly with a dermatological freezing spray (Kälte 75; Kontakt Chemie, Rastatt, FRG) and then incubated in distilled water of 25° C. After 5 min the shrunken segment was extended to its original length by applying a force F_1 = 0.196 N (corresponding to 20 *g*; this force was found to compensate for the shrinkage caused by turgor loss of the killed tissue in all experiments). After 5 min an additional force F_2 of 0.196 N was applied, removed again after 15 min, and reapplied after a further 5 min. E_{tot}, E_{el}, and E_{pl} were graphically determined as indicated

Results

1. Limiting conditions of measuring in-vitro extensibility. Both E_{el} and E_{pl} are linearly related to the stretching force applied to frozen-thawed coleoptile segments in the apparatus shown in Fig. 1 up to at least 0.392 N (40 g) of weight (Fig. 3). Since the segments were extended to their original length before measurement (Fig. 2), E_{el} includes only a small part of the total elastic extension in a range in which the elastic modulus appears to be approximately constant. Throughout the following experiments a standard force of 0.196 N (20 *g*) was chosen for in-vitro extensibility measurements. Figure 4a shows typical recorder tracings obtained by segments after incubation on water for 1 h (time zero in the growth experiments) and after a subsequent 6-h treatment with IAA or IAA+ABA. These in-vitro extension kinetics (E_{tot}) can be fitted by straight lines if the extension (deformation) is plotted vs. log time (Fig. 4b), indicating that the cell walls exhibit viscoelastic properties (Cleland 1971b, 1981; Ferry 1980). The steepness of these lines, extrapolating to a common point on the abscissa, represents the wall extensibility. For practical reasons, however, the extent of deformation after a constant stretching period of 15 min (operational unit: μm·20 g^{-1}·15 min^{-1}), rather than steepness, was used as a relative measure of E_{tot} and its components E_{el} and E_{pl} as described in Fig. 2. Later it will be seen that pysiologically induced changes of creep (E_{tot}) can be accounted for quantitatively by changes of E_{pl}.

Figures 3 and 4 show that the practically non-growing segments obtained after 1 h of water incubation (Kutschera and Schopfer 1985a) demonstrate a rather large E_{pl} approaching about 50% of the value of segments rapidly growing in the presence of IAA. Similar results have been found in previous investigations (e.g. Cleland 1967). It is obvious that this initial in-vitro extensibility cannot be related to growth. Initial values of E_{tot} (95 μm·20 g^{-1}·15 min^{-1}), E_{el} (40 μm·20 g^{-1}·

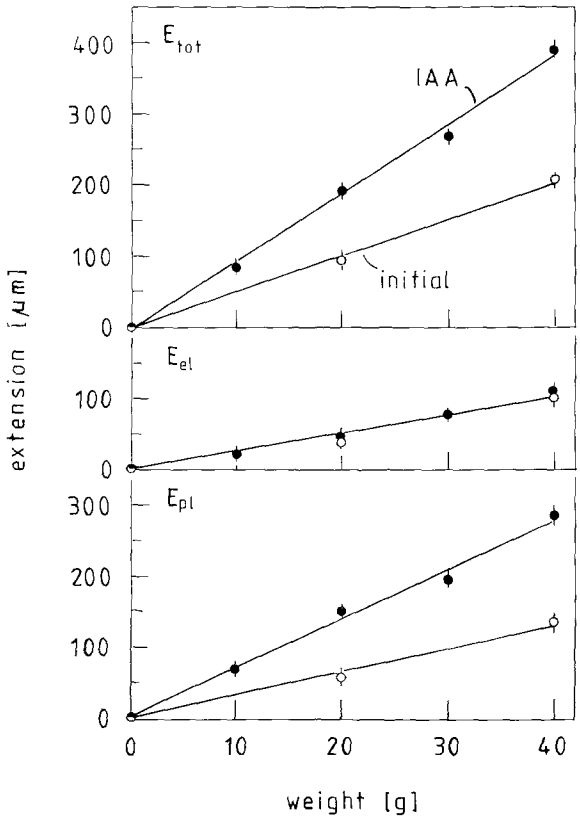

Fig. 3. The dependence of E_{tot}, E_{el}, and E_{pl} on applied force. Nonabraded segments were either kept on water for 1 h after cutting (o—o) or additionally incubated for 6 h in IAA (6 µmol·l^{-1}, ●—●). In-vitro extension was measured at variable force (F_2, expressed in terms of the mass of weights applied to the segments) as indicated in **Fig. 2.** Fresh segments were used for each measurement

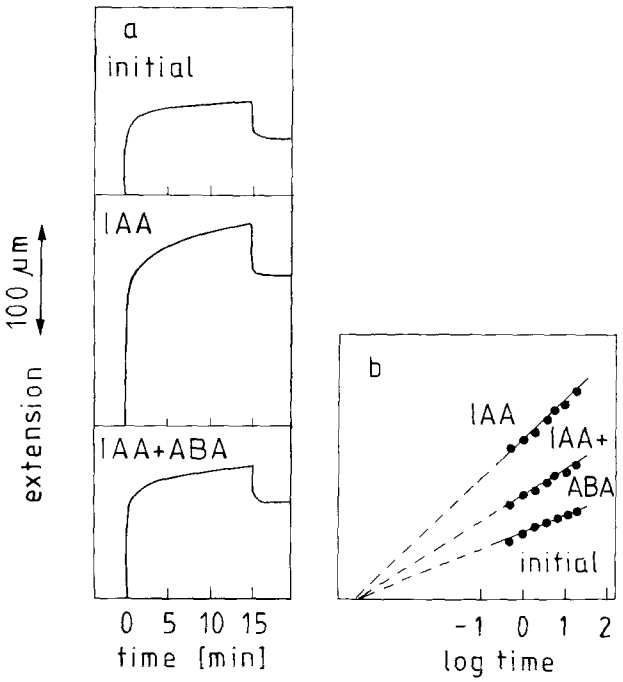

Fig. 4. a In-vitro extension kinetics measured (as described in **Fig. 2**) with nonabraded segments incubated, after cutting, for 1 h on water (initial), 1 h on water + 6 h on IAA (6 µmol·l^{-1}), or 1 h on water + 6 h on IAA (6 µmol·l^{-1}) and ABA (0.25 mmol·l^{-1}). **b** Semi-log plots of the same curves during the stretching period (0–15 min)

15 min^{-1}), and E_{pl} (54 µm·20 g^{-1}·15 min^{-1}) have, therefore, been subtracted for assessing actual extensibilities utilized in cell elongation in vivo. Thus, increases of exensibility (ΔE_{tot}, ΔE_{el}, ΔE_{pl}) rather than absolute extensibilities are related to growth rates throughout in the following experiments.

2. Effect of IAA on elongation rate and in-vitro extensibility. Figure 5 shows the elongation kinetics of segments in the presence of an optimal and a subthreshold concentration of IAA. The in-vitro extensibility of single segments taken from these experiments at appropriate times was measured. While ΔE_{el} only slightly increases in the presence or absence of IAA, there is a strong IAA-dependent increase of ΔE_{pl}, rising after a lag of about 15 min (so does elongation, see Fig. 3 in Kutschera and Schopfer 1985a) at the high IAA concentration. The time course of elongation rate calculated from the elongation kinetics was fitted to the time course of ΔE_{pl} using the 3-h point for normalization. Theoretically, a strict quantitative correlation between elongation rate and in-vitro extensibility would be expected if ΔE_{pl} truly represented m (and if m was determining the growth rate). Figure 5 shows that this expectation is actually borne out with the exception of the last 2 h where the ΔE_{pl} curve continues with constant rate while the elongation rate significantly decreases. Such a deviation is to be expected since the growth rate depends also on osmotic pressure, which decreases significantly after some hours of growth due to the uptake of water (Table 1). The limited extensibility measurements of water-control segments also fit into the correlation between growth rate and ΔE_{pl}.

3. Effect of ABA on elongation rate and in-vitro extensibility. Figure 6 shows the kinetics of ABA inhibition of elongation in IAA-treated maize coleoptile segments, together with the concomitantly measured changes of in-vitro cell wall extensibility. Except at later stages (>3–4 h after initiation of growth by IAA), ABA decreases elongation rate and ΔE_{pl} in a closely correlated manner if the inhibitor is applied either together with IAA or after establishment of a steady-state growth response. A preincubation with ABA for 1 h has no additional effect, indicating that ABA specifically af-

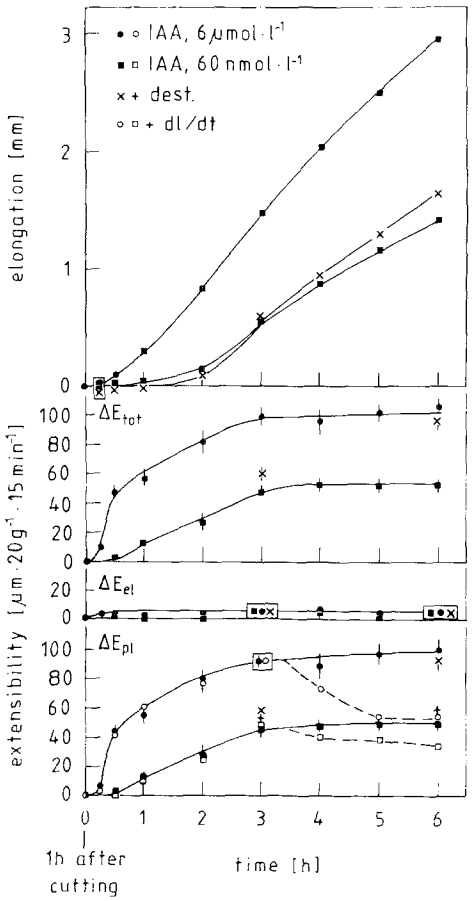

Fig. 5. Kinetics of elongation (length increase per segment) and in-vitro extensibility (ΔE_{tot}, ΔE_{el}, ΔE_{pl}) in nonabraded segments incubated in 6 µmol·l^{-1} (●—●) or 60 nmol·l^{-1} (▪—▪) IAA (×—×, water control). The elongation curves were differentiated graphically and the calculated elongation rates (○, □, +) were fitted to the ΔE_{pl} kinetics using the point at 3 h IAA (6 µmol·l^{-1}) for normalization

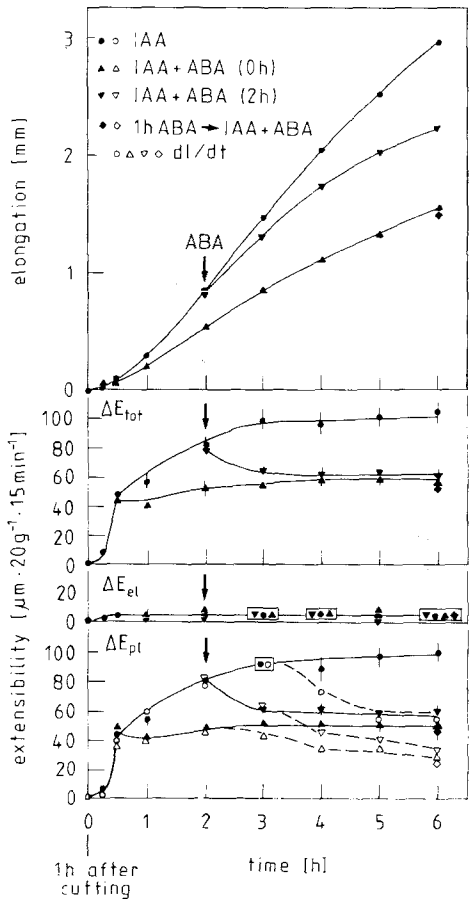

Fig. 6. Effect of ABA on the kinetics of IAA-mediated elongation and in-vitro extensibility (ΔE_{tot}, ΔE_{el}, ΔE_{pl}) in nonabraded segments (water controls as in **Fig. 5**). ABA (0.25 mmol·l^{-1}) was applied either together with IAA (6 µmol·l^{-1}) at time zero (▲—▲), 1 h before IAA (◆—◆), or 2 h after IAA (▼—▼). Elongation rates (*open symbols*) were calculated as in **Fig. 5**

Table 1. Changes of cell sap osmolality during elongation of nonabraded segments in the presence of IAA (6 µmol·l^{-1}), and ABA (0.1 mmol·l^{-1}, in the presence of 6 µmol·l^{-1} IAA). The medium was distilled water

Treatment (1 h after cutting)	Segment length (mm)	Osmolality (mosmol·kg^{-1})
Initial (0 h)	10.0±0.0	277±3
4 h IAA	12.2±0.1 (+22%)	204±5 (−26%)
8 h IAA	14.2±0.1 (+42%)	172±2 (−38%)
4 h IAA+ABA	11.0±0.1 (+10%)	248±3 (−10%)
8 h IAA+ABA	12.1±0.1 (+21%)	214±2 (−23%)

fects cell wall extension rather than inhibiting metabolism in general. In agreement with this result, there is no detectable influence of ABA (and IAA) on protein and RNA synthesis (Fig. 7). Oxygen consumption is likewise not affected by ABA and IAA, at least up to 6 h after hormone application (Kutschera 1985). Table 1 shows that segment lengthening in the presence of IAA or IAA+ABA is accompanied by an inverse change of the solute concentration of the cell sap, i.e. the cells behave like ideal osmometers (Zimmermann 1978). In other words, the observed changes of osmolality can quantitatively be related to the respective water uptake caused by volume growth, indicating that ABA and IAA have no direct effect on the osmotic state of the tissue.

The results of Fig. 6 prompt the question whether ABA specifically interferes with the mechanism by which IAA promotes wall loosening, or if it counteracts the IAA response by an independent wall-stiffening mechanism. Figures 8 and 9 provide evidence that ABA does in fact not interfere directly with the action of IAA, since it also inhibits growth and ΔE_{pl} increase elicited by exogenous protons (buffer at pH 4) or endogenous protons (excreted by an FC-activated proton pump). It was recently shown that acid (pH < 5)

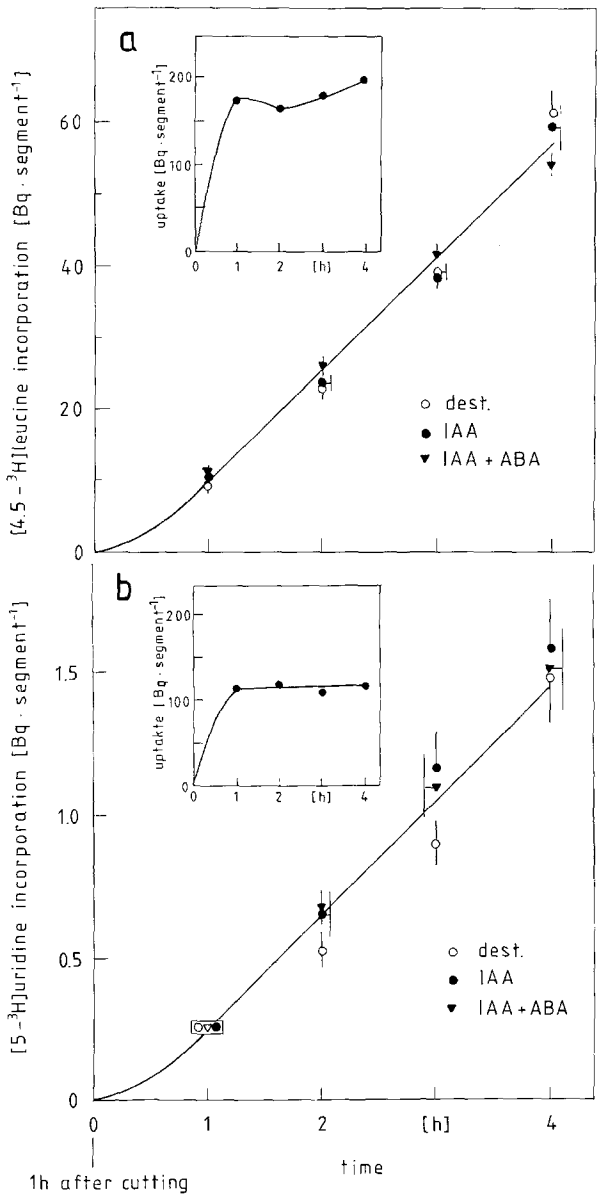

Fig. 7. Effect of IAA (6 μmol·l^{-1}) and ABA (0.1 mmol·l^{-1}, in the presence of 6 μmol·l^{-1} IAA) on the incorporation of [^{3}H]leucine (**a**) and [^{3}H]uridine (**b**) into acid-precipitable material of nonabraded segments. Hormones and tracers were applied together at time zero. The *insets* show the time courses of tracer uptake into the tissue in the presence of IAA

and IAA induce cell growth by unrelated mechanisms (Kutschera and Schopfer 1985a, b). The inhibitory effect of ABA on acid-induced growth was previously demonstrated in *Avena* coleoptile segments (Rehm and Cline 1973b). However, it has not been possible to decide in these experiments if ABA acts by inhibiting cell wall loosening or by lowering the turgor pressure. Figure 8 confirms the results of Rehm and Cline (1973b) and additionally shows that ABA interferes with acid-induced cell wall loosening, extending results of Van

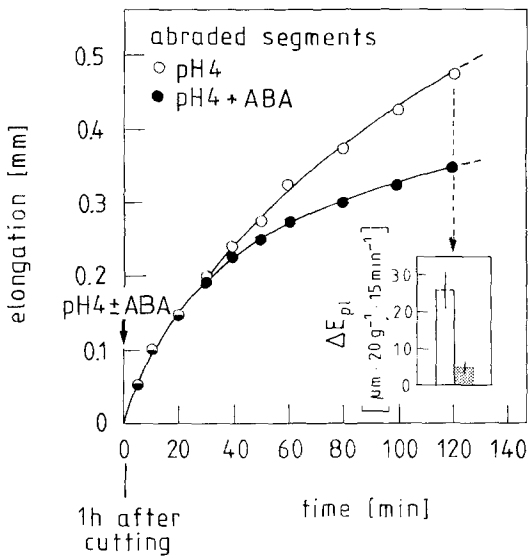

Fig. 8. Effect of ABA on acid-induced elongation and ΔE_{pl} (after 2 h, *inset*) in abraded segments. Buffer (citric acid/Na-citrate, pH 4, 7 mmol·l^{-1} citrate) with or without ABA (0.1 mmol·l^{-1}) was applied at time zero. There was no effect of ABA or buffer on ΔE_{el}

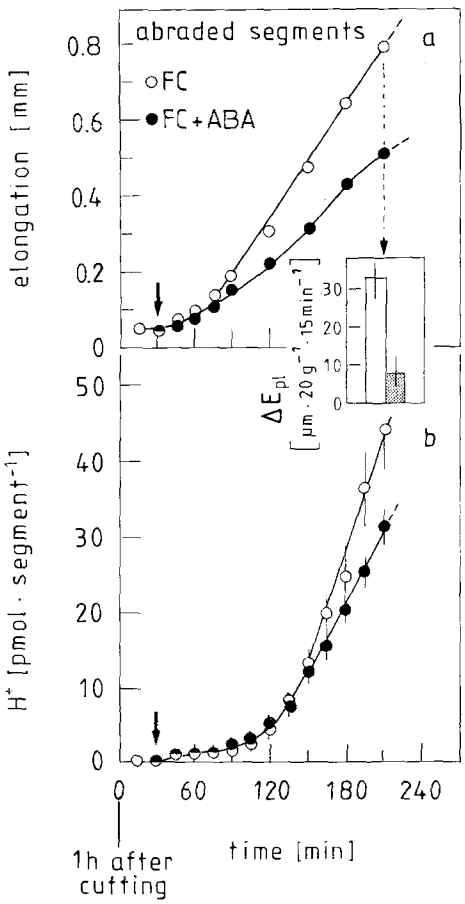

Fig. 9. Effect of ABA on FC-induced elongation (**a**), ΔE_{pl} (after 3 h, inset) and H^+ excretion (**b**). Abraded segments were incubated in 0.1 mmol·l^{-1} $Ca(OH)_2$ pH 7, at time zero. FC (1 μmol·l^{-1}) with or without ABA (0.1 mmol·l^{-1}) was added after 30 min. There was no effect of ABA or FC on ΔE_{el}

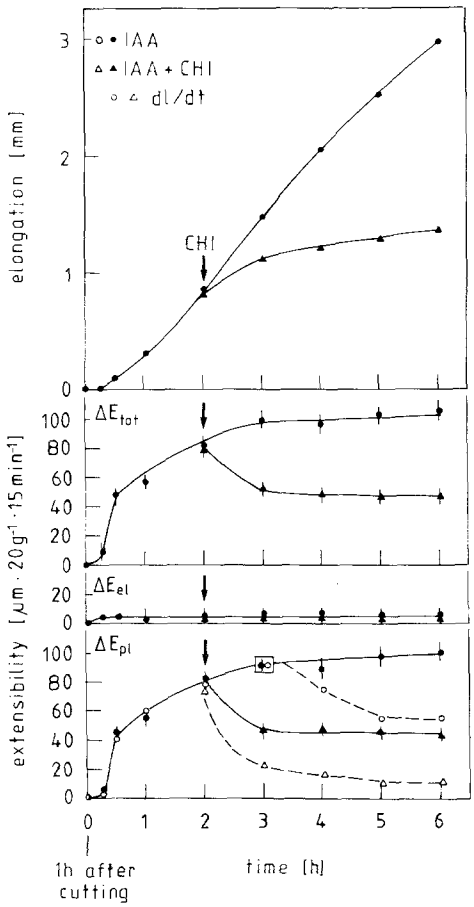

Fig. 10. Effect of CHI on the kinetics of IAA-mediated elongation and in-vitro extensibility (ΔE_{tot}, ΔE_{el}, ΔE_{pl}) in nonabraded segments. CHI (10 mg·l^{-1}) was applied 2 h after IAA (6 μmol·l^{-1}). Elongation rates (*open symbols*) were calculated as in **Fig. 5**

Table 2. Changes of cell sap osmolality during elongation of nonabraded segments in the presence of IAA (6 μmol·l^{-1}) with or without CHI (10 mg·l^{-1}). The medium was distilled water

Treatment (1 h after cutting)	Segment length (mm)	Osmolality (mosmol·kg^{-1})
Initial (0 h)	10.0	261±2
2 h IAA	10.8±0.1 (+ 8%)	235±3 (−10%)
6 h IAA	13.1±0.1 (+31%)	186±2 (−29%)
2 h IAA + 2 h (IAA+CHI)	11.1±0.1 (+11%)	211±3 (−19%)
2 h IAA + 4 h (IAA+CHI)	11.2±0.1 (+12%)	213±2 (−18%)

Volkenburgh and Davies (1983) who reported that a pretreatment with ABA lowers the capacity for acid-induced wall loosening in leaf strips of *Phaseolus vulgaris*. Similarly, the growth-promoting effect of a high concentration of FC, mediated by endogenous cell wall acidification (Kutschera and Schopfer 1985b) can be inhibited by ABA and is also attributable to a reduction of wall extensibility (Fig. 9). In these experiments there is only a slight inhibition of FC-induced H^+ excretion by ABA. This inhibition is not detectable before approx. 60 min after the inhibition of segment elongation; therefore, it cannot be causally involved in the effect of ABA on ΔE_{pl}. Similar experiments (Kutschera 1985) with IAA (6 μmol·l^{-1}) have shown that auxin-induced H^+ excretion is not influenced either by ABA during the first 2 h of treatment. [Subsequently there is a significant inhibition of H^+ excretion, as previously reported for *Avena* coleoptiles by Rayle (1973).]

In order to allow penetration of protons through the cuticle, the surface of the segments had to be abraded in the experiments of Figs. 8 and 9 (Kutschera and Schopfer 1985a). This treatment decreases growth rates and ΔE_{pl} by about 30%.

4. Effect of CHI on elongation rate and in-vitro extensibility. Coleoptile growth mediated by IAA very rapidly responds to protein-synthesis inhibitors such as CHI (Rehm and Cline 1973a; Bates and Cleland 1979; Kutschera and Schopfer 1985b). We have investigated whether this growth inhibition can also be quantitatively related to a concomitant decrease of in-vitro extensibility. Figure 10 shows that CHI (10 mg·l^{-1}) rapidly decreases both ΔE_{pl} and elongation rate. However, while this CHI concentration inhibits the long-term elongation rate by 80–90%, ΔE_{pl} is decreased by about 50% only. This finding confirms Cleland's (1970) results with *Avena*, equally stating that CHI inhibits cell elongation more strongly than in-vitro extensibility. It thus appears that the effect of CHI on IAA-mediated growth cannot exclusively be attributed to a reduction of ΔE_{pl}. As shown in Table 2, CHI reduces cell sap osmolality about twice as much as expected from growth-dependent water uptake, indicating that this inhibitor causes some leakage of osmotic material from the tissue.

Discussion

In the present study we have found a close correspondence between growth rate, controlled by IAA or ABA, and in-vitro cell wall extensibility (ΔE_{tot}), as measured with the creep test, over a period of 3–4 h. The elastic extensibility was not affected by these agents, supporting the notion that it is exclusively the plastic component (ΔE_{pl}) of total extensibility, measured as creep, that is affected by growth hormones. The discrepancies between growth rate and ΔE_{pl} observed after more than 4 h in Figs. 5

and 6 can be explained by a diminution of the growth rate at constant cell wall extensibility as a result of reduced turgor pressure. It is well established (see Table 2) that the osmotic pressure of the cell sap, and thus the turgor, will fall during growth in the absence of absorbable solutes. Hence the growth rate will decrease after some time even if m remains constant. As expected, this effect can be eliminated by including sucrose or KCl in the incubation medium (Cleland 1967; Stevenson and Cleland 1981). Thus we may conclude that the differences between elongation rates and ΔE_{pl} beyond 4 h in Figs. 5 and 6 are not caused by discrepancies between ΔE_{pl} and m, but result from the lack of a constant relationship between m and elongation rate. Taking this into account, our results do not support the hypothesis of Vanderhoef and Dute (1981) that IAA has – in addition to stimulating wall loosening – a second, mechanistically different function which would be necessary to sustain long-term growth, temporally separated from the wall-loosening response.

Concerning ABA, we can conclude from our data (Fig. 6, Table 1) that the growth-inhibiting effect of this hormone is mediated by an inhibition of cell wall loosening rather than by a reduction of turgor pressure. Since a similar conclusion has been reached in the case of germinating seeds (Schopfer and Plachy 1985), we propose that ABA generally inhibits cell growth by rendering the cell wall more rigid and, consequently, more resistant to the expanding forces of turgor pressure. Under natural conditions, this mechanism may be of some importance during periods of water stress, where cell wall loosening in growing leaves etc. would greatly augment wilting. A reversible inhibition of cell growth by stress-induced ABA provides an effective possibility, in addition to stomata closure, of saving water for the maintenance of turgor pressure. The biochemical mechanism of ABA-mediated cell wall stiffening is not clear yet. We do know, however, that ABA is functioning in the same way, irrespective of whether the wall structure is loosened by either IAA or low pH. This excludes the possibility of ABA acting directly as a competitive antagonist of IAA. Furthermore, proton transport at the plasma membrane does not seem to play a role in the action mechanism of ABA (Fig. 9).

The quantitative correlation between growth rate and ΔE_{pl} in our experiments rests on two assumptions: (i) The in-vitro extensibility of the untreated, nongrowing tissue (initial value of E_{pl}, see Fig. 4) is irrelevant to cell extension in vivo; (ii) this basal level of extensibility is constant during the experimental period of 6 h. As proposed in principle by Cleland (1971 b), we assume that the tissue subjected to unidirectional stress during the creep test converts a constant amount of plasticity into extension which is not utilized under multidirectional stress (turgor pressure). Consequently, only the amount of plasticity exceeding this basal level represents the extensibility which is usable for elongation growth. Indirect evidence supporting this view has been provided by Cleland (1970) who has shown that CHI has no effect on the basal extensibility of *Avena* coleoptile segments over at least 6 h although the IAA-induced increment of in-vitro extensibility and cell elongation are strongly reduced. Thus, even though definite proof for the above assumptions is presently not available yet, they provide a reasonable basis for an empirical analysis of in-vitro extensibility measurements. Recent measurements of creep in living maize coleoptile segments (to be published elsewhere) led to extensibility kinetics which are very similar to those obtained with frozen-thawed material. This shows that the cell walls of the killed segment, pre-extended to their in-vivo length before measurement, are comparable in their rheological properties to the walls of the undisturbed, living segment extended by turgor pressure.

The conclusion that in-vitro extensibility measurements do not represent the in-vivo extensibility m has mainly been based on kinetic differences between growth rate and the "Instron extensibility" changes of denatured (methanol- and pronase-treated) cell walls of *Avena* coleoptile segments. In-vitro extensibility changes produced by IAA or growth-inhibiting agents have been considered too small, or too late, compared with the actual growth rate changes and, therefore, have been attributed to an average value of m over some previous period of time (60–90 min in *Avena* coleoptile) rather than to the instantaneous value of m (e.g. Cleland 1967, 1984). Our results show that this view may not be generally true. If ΔE_{pl} really included wall-loosening events from a previous period of time, one would expect a transitory discrepancy between ΔE_{pl} and growth rate with a maximum immediately after the induced growth change. In maize coleoptiles analyzed with the creep test after freeze-killing we have found no evidence for such a time lag between growth changes and in-vitro extensibility changes both in step-up (IAA) and in step-down (ABA) experiments (Figs. 5, 6). Rather, the kinetics of E_{pl} changes elicited by both IAA and ABA closely follow the corresponding growth-rate changes over several hours, and there is therefore no reason to doubt

that ΔE_{pl} represents a reliable measure of m. Also in the case of growth inhibition by CHI, there is no indication for a temporal displacement between growth-rate change and ΔE_{pl}, although steady-state growth is disproportionately reduced (Fig. 10). In order to resolve the controversial results of the different laboratories it would be necessary to compare the various experimental systems by measuring the kinetics of growth and in-vitro extensibility simultaneously, under strictly standardized conditions.

In contrast to ABA, CHI inhibits cell elongation more strongly than expected from its effect on ΔE_{pl} (Fig. 10). Although CHI does lead to some leakage of solutes (Table 2), this effect is obviously too small to explain quantitatively the discrepancy between steady-state growth rate and ΔE_{pl} observed in these experiments. We must, therefore, conclude that CHI inhibits cell elongation not only by reducing m (ΔE_{pl}) and osmotic pressure but also by additionally affecting another growth parameter.

We are greatly indebted to Dr. B. Lercari (Pisa) for a sample of fusicoccin and Dr. R.E. Cleland (Seattle) for helpful discussions. The work was supported by the Deutsche Forschungsgemeinschaft (SFB 206).

References

Bates, G.W., Cleland, R.E. (1979) Protein synthesis and auxin-induced growth: inhibitor studies. Planta **145**, 437–442

Cleland, R.E. (1967) Extensibility of isolated cell walls: measurement and changes during cell elongation. Planta **74**, 197–209

Cleland, R.E. (1970) Protein synthesis and wall extensibility in the *Avena* coleoptile. Planta **95**, 218–226

Cleland, R.E. (1971a) Cell wall extension. Annu. Rev. Plant Physiol. **22**, 197–222

Cleland, R.E. (1971b) The mechanical behavior of isolated *Avena* coleoptile walls subjected to constant stress. Properties and relation to cell elongation. Plant Physiol. **47**, 805–811

Cleland, R.E. (1981) Wall extensibility: hormones and wall extension. In: Encyclopedia of plant physiology, N.S., vol. 13b: Plant carbohydrates II. Extracellular carbohydrates, pp. 255–273, Tanner, W., Loewus, F.A., eds. Springer, Berlin Heidelberg New York

Cleland, R.E. (1984) The Instron technique as a measure of immediate-past wall extensibility. Planta **160**, 514–520

Deschamp, P.A., Cooke, T.J. (1984) Causal mechanisms of leaf dimorphism in the aquatic angiosperm *Callitriche heterophylla*. Am. J. Bot. **71**, 319–329

Ferry, J.D. (1980) Viscoelastic properties of polymers, 3th edn, John Wiley, New York

Gulati, D.K., Rosenthal, G.A., Sabharwal, P.S. (1979) An improved method for estimating macromolecular synthesis. J. Exp. Bot. **30**, 919–924

Heyn, A.N.J. (1931) Der Mechanismus der Zellstreckung. Rec. Trav. Bot. Neerl. **28**, 113–244

Heyn, A.N.J. (1933) Further investigations on the mechanism of cell elongation and the properties of the cell wall in connection with elongation. Protoplasma **19**, 78–96

Kutschera, U. (1985) Untersuchungen zum Wirkungsmechanismus von Auxin und Abscisinsäure bei der Regulation des Zellstreckungswachstums von Koleoptilsegmenten (*Zea mays* L.). Dissertation, University of Freiburg

Kutschera, U., Schopfer, P. (1985a) Evidence against the acid-growth theory of auxin action. Planta **163**, 483–493

Kutschera, U., Schopfer, P. (1985b) Evidence for the acid-growth theory of fusicoccin action. Planta **163**, 494–499

Masuda, Y. (1978) Auxin-induced cell wall loosening. Bot. Mag., Spec. Issue **1**, 103–123

Penny, D., Penny, P., Marshall, D.C. (1974) High resolution measurement of plant growth. Can. J. Bot. **52**, 959–969

Philipson, J.J., Hillman, J.R., Wilkins, M.B. (1973) Studies on the action of abscisic acid on IAA-induced rapid growth of *Avena* coleoptile segments. Planta **114**, 87–93

Rayle, D.L. (1973) Auxin-induced hydrogen-ion secretion in *Avena* coleoptiles and its implications. Planta **114**, 63–73

Rehm, M.M., Cline, M.G. (1973a) Rapid growth inhibition of *Avena* coleoptile segments by abscisic acid. Plant Physiol. **51**, 93–96

Rehm, M.M., Cline, M.G. (1973b) Inhibition of low pH-induced elongation in *Avena* coleoptiles by abscisic acid. Plant Physiol. **51**, 946–948

Schopfer, P., Plachy, C. (1985) Control of seed germination by abscisic acid. III. Effect on embryo growth potential (minimum turgor pressure) and growth coefficient (cell wall extensibility) in *Brassica napus* L. Plant Physiol. **77**, 676–686

Stevenson, T.T., Cleland, R.E. (1981) Osmoregulation in the *Avena* coleoptile in relation to auxin and growth. Plant Physiol. **67**, 749–753

Taiz, L. (1984) Plant cell expansion: regulation of cell wall mechanical properties. Annu. Rev. Plant Physiol. **35**, 585–657

Vanderhoef, L.N., Dute, R.R. (1981) Auxin-regulated wall loosening and sustained growth in elongation. Plant Physiol. **67**, 146–149

Van Volkenburgh, E., Davies, W.J. (1983) Inhibition of light-stimulated leaf expansion by abscisic acid. J. Exp. Bot. **34**, 835–845

Zenk, M.H. (1970) Phytohormone und Genaktivität. Ber. Dtsch. Bot. Ges. **83**, 325–344

Zimmermann, U. (1978) Physics of turgor- and osmoregulation. Annu. Rev. Plant Physiol. **29**, 121–148

Received 8 October; accepted 4 December 1985