J Plant Growth Regul (1993) 12:13–19

Journal of
Plant
Growth
Regulation

Effects of Abscisic Acid Analogues on Abscisic Acid-Induced Gene Expression in Barley Aleurone Protoplasts: Relationship Between Structure and Function of the Abscisic Acid Molecule

R. M. Van der Meulen,[1] F. Heidekamp,[1] B. Jastorff,[2] R. Horgan,[3] and M. Wang[1]*

[1]Centre for Phytotechnology RUL-TNO, Department of Molecular Plant Biotechnology, Leiden, The Netherlands; [2]Department of Chemistry, Bioorganic Chemistry Unit, University of Bremen, Bremen, Germany; and [3]Department of Biological Sciences, The University College of Wales, Aberystwyth Dyfed, UK

Received September 4, 1992; accepted November 5, 1992

Abstract. The plant hormone abscisic acid (ABA) mediates gene expression in barley aleurone protoplasts. In order to elucidate the essential functional groups of the ABA molecule, the specificity of a number of ABA analogues for inducing ABA-regulated gene (e.g., RAB, BASI) expression in barley aleurone protoplasts was studied. These analogues have modifications at three different positions of the ABA molecule: (a) the 1′-hydroxyl group (1′-deoxy ABA), (b) the carboxyl group (ABA-methyl ester or ABA-glucose ester), and (c) both the 1′-hydroxyl and 4′-carbonyl groups (α-ionylidene acetic acid). The importance of the different putative functional groups was analyzed. The dose–response analysis of ABA analogues upon the induction gene expression showed the following order: ABA > ABA methyl ester > 1′-deoxy ABA > ABA glucose ester > α-ionylidene acetic acid > α-β-ionone.

The phytohormone abscisic acid (ABA) mediates the response of plants to osmotic stress and plays an important role in embryogenesis and grain development (Dure et al. 1981, Skriver and Mundy 1990). During germination, ABA counteracts the effects of gibberellin (GA_s) on the induction of germination (Allen and Trewavas 1987, Black 1983, Hepler and Wayne 1985, Walker-Simmons 1987, Walton 1987). It has been reported that ABA is able to induce both cytosolic pH and Ca_i level changes (Gehring et al. 1990, Van der Veen et al. 1992, Wang et al. 1991, 1992). The multiple responses to ABA have been observed in various plants and plant tissues. However, little is known about the signal recognition and transduction.

A number of plant genes have been cloned which are abundantly expressed during late embryogenesis. Some of these genes are ABA-responsive and some of them can be induced by osmotic stress, salt stress, and desiccation, etc. (Skriver and Mundy 1990). The RAB gene family belongs to this type of gene (Skriver and Mundy 1990). Another plant hormone, GA_s, plays an important role during germination in cereals. It is known that a group of hydrolase mRNAs are induced by GA_s. At this time, ABA acts as an antagonist of the effects of GA_s on the various cellular functions (Nolan and Ho 1988, Rogers 1988). ABA not only inhibits the expression of GA_s-responsive hydrolase mRNA and protein, but also promotes the accumulation of a protein which inhibits germination-specific α-amylase, such as bifunctional α-amylase subtilisin inhibitor (BASI) (Leah and Mundy 1989, Mundy and Rogers 1986).

Most research concerning the mechanism of ABA action has been focused on the expression of ABA-regulated genes and promoters. On the other hand, ABA is able to trigger a series of cellular responses which occur long before ABA-induced gene expression can be observed (Gehring et al. 1990, Van der Veen et al. 1992, Wang et al. 1991). It is presumed that ABA must interact in a structure-specific manner with a receptor protein as the first step of its mechanism of action. The binding of ABA to a putative receptor has been reported by Hornberg and Weiler (1984). Unfortunately, there have been no confirmations of this important observation. It has been shown that structural modifications of the natural hormone (ABA analogues) results in a reduction of biological activity (Milborrow

* Author for correspondence

1986, Nagano et al. 1980, Schubert et al. 1991, Walker-Simmons et al. 1991, 1992, Walton 1983). Any modification that disturbs one or more essential interactions between ABA and a correspondent amino acid at a hormone receptor will drastically reduce the affinity of an analogue or even lead to loss of activity (Schubert et al. 1991, Walton 1983). In order to ascertain which structural features of the ABA molecule may be involved in its interaction with a possible receptor protein, we have investigated the dose–response of induction of RAB-16 and BASI mRNA by various functional group analogues of ABA.

Materials and Methods

Materials

(R,S)-ABA (chemical purity 99.9%), (R,S)-ABA methyl ester (chemical purity 99.9%), (R,S)-α-ionone, and (R,S)-β-ionone (chemical purity 99.9%) were obtained from Sigma Chemicals Co. (St. Louis, Missouri, USA). (R,S)-ABA glucose ester (chemical purity 98.9%) was from Apex Organic LTD (Cambridge, UK). (R,S)-1′-deoxy-ABA and (R,S)-α-ionylidene acetic acid (chemical purity 99%) were synthesized by R. Horgan. Cellulase Onozuka R-10 was obtained from Yakult Honsha (Tokyo, Japan) and Gamborg B5 from Flow Laboratories (Irvine, UK). PVP K25 was from Fluka Chemie (Switzerland) and PIPES from Janssen Chemical (Tilburg, The Netherlands). [α-^{32}P]-dCTP was obtained from Amersham Int. (UK). All other chemicals were from Merck (Darmstadt, Germany).

Isolation of Protoplasts

Barley (*Hordeum vulgare* L. cv. Himalaya, harvest 1985; Department of Agronomy, Washington State University, Pullman, Washington, USA) aleurone protoplasts were prepared as previously reported (Wang et al. 1991, 1992). Protoplasts were washed three times with washing buffer (0.5 M mannitol, 10 mM KCl, 1 mM $MgCl_2$, 1.1 mM $CaCl_2$, 0.1 mM EGTA, 0.5 mM K_2HPO_4, 10 mM PIPES-HCl (pH 6.8).

RNA Isolation and Analysis

Barley aleurone protoplasts (4×10^5/ml) were incubated with different ABA analogues in the dark at 22°C for 2.0 h. ABA-induced mRNA expression was analyzed by Northern analysis as follows. Total cellular RNA from 2×10^6 protoplasts was isolated and purified as previously described (Wang et al. 1992). Northern blots were made by separating the RNA on a glyoxal/DMSO 1% agarose gel (Sambrook et al. 1989) and subsequent blotting onto a nylon membrane as suggested by the manufacturer (GeneScreen Plus, DuPont, Pennsylvania, USA). Northern transfers were hybridized at 65°C in 1% SDS, 1 M NaCl, 10% dextran sulphate, and 0.1 mg/ml sonificated salmon sperm DNA to cDNA probes (a RAB-16 cDNA probe from rice and a BASI cDNA probe from barley), which were labeled with [α-^{32}P]dCTP by using random primers. After hybridization, the blots were washed at 65°C, twice in 2 × SSC, twice in 2 × SSC + 1% SDS, and finally in 0.1 × SSC. The amount of ^{32}P-labeled cDNA probes hybridized to specific mRNA was estimated by densitometry (Ultroscan KL densitometer, LKB).

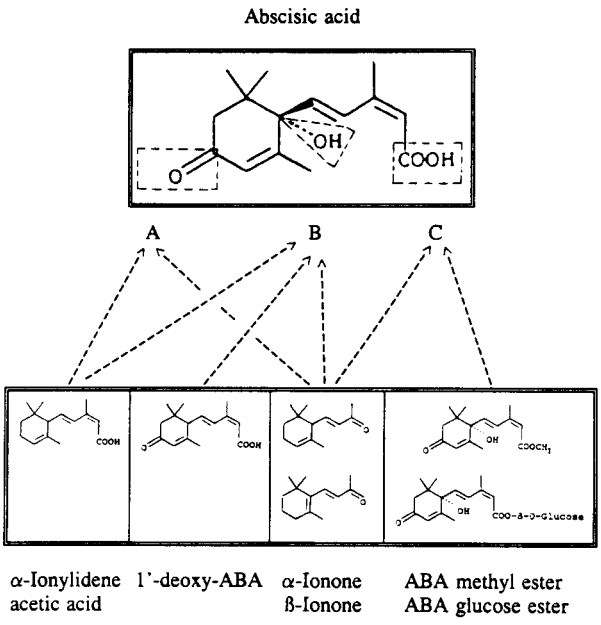

Fig. 1. Structure of abscisic acid, and its analogues: **(A)** modification on carbonyl group; **(B)** modification on hydroxyl group, and **(C)** modification on carboxyl group.

ABA Measurement and TLC Analysis

ABA extraction was carried out as described by Walker-Simmons (1988). Samples were analyzed by precoated silica gel plates (60 F_{254}, Merck). For TLC analysis, the solvent system was chloroform/methanol/water (75:22:3 vol/vol). ABA or ABA analogues could be detected as a quenching spot under UV light (Dumbroff et al. 1983). The spots on TLC plates, where according to the R_f value the ABA should be, were scratched off and dissolved in 200 µl 96% ethanol. Subsequently, the absorbency at 240 nm of these solutions was measured in a spectrophotometer (U-2000, Hitachi, Japan).

Results

Rationale for Selection of ABA Analogues

In our choice of analogues we sought to eliminate or change the possible interactions of polar groups in the ABA molecule (Fig. 1) with a putative receptor. While the interactions may involve the formation of hydrogen bonds, the presence or absence of certain functional groups may also involve conformation changes in the ABA molecule, which affect its attachment to a receptor.

The negative charge of the carboxyl group is removed in the methyl and glucose esters of ABA, which are still capable of hydrogen-bond to a potential receptor. The 1′-hydroxyl group, and thus a strong hydrogen bond donor potential, is removed in 1′-deoxy-ABA. In α-ionylidene acetic acid, both the 1′-hydroxyl and the 4′-carbonyl group (a hydrogen bond acceptor) are missing. In α- and β-ionone,

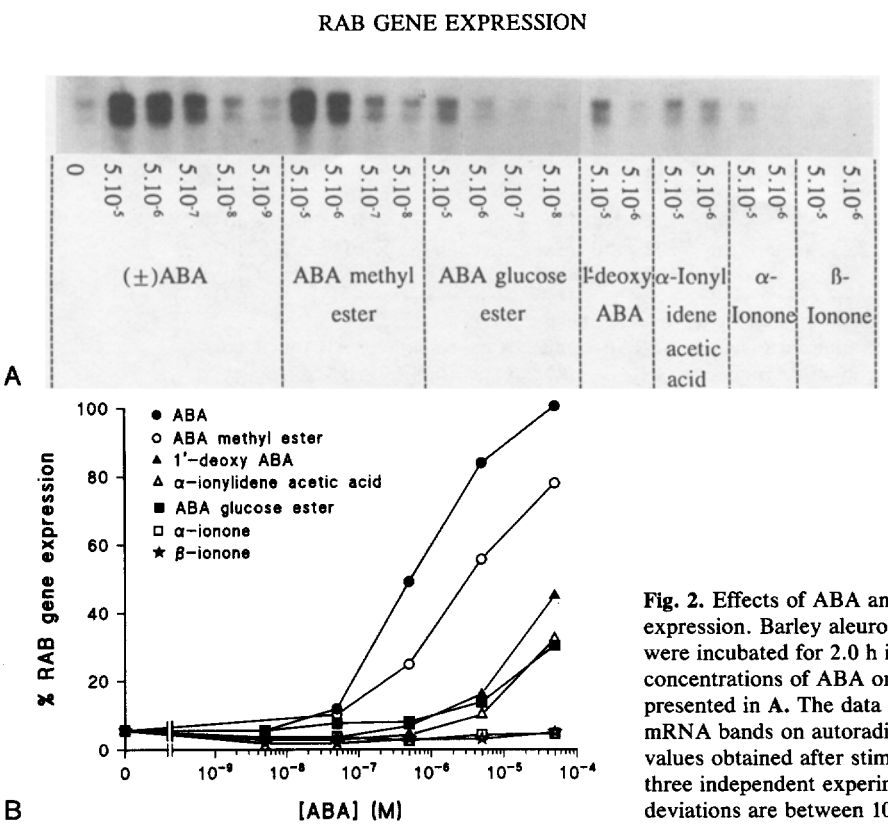

Fig. 2. Effects of ABA and ABA analogues on RAB-16 mRNA expression. Barley aleurone protoplasts (4×10^5 protoplasts/ml) were incubated for 2.0 h in the presence of different concentrations of ABA or ABA analogues. A typical example is presented in **A**. The data represent relative OD values of RAB-16 mRNA bands on autoradiograms expressed as percentage of values obtained after stimulation at 50 μM ABA (**B**). The mean of three independent experiments is presented. The standard deviations are between 10% and 15%.

three presumable interacting groups have been changed or removed (i.e., -COOH is replaced by -CHO, -OH is replaced by -H, and -C=O is replaced by $-CH_2$ respectively).

The commercial ABA analogues used were of high chemical purity (>99%). The synthetic ABA analogues were unlikely to be contaminated with ABA since they were synthesized de novo and not via ABA; therefore, no side reactions were possible which might give rise to ABA (unpublished data). In addition, TLC analysis showed that all the ABA analogues contained less than 0.01% ABA. ABA and all ABA analogues which we used were the racemic mixture of R and S optical isomers. Thus, the data from the optical purity point of view are comparable.

Effects of ABA Analogues on Gene Expression

To elucidate which of the putative functional groups of the ABA molecule are involved in signal transduction leading to changes in gene expression, the ABA-analogues were used to investigate the induction of both RAB-16 and BASI mRNA.

Generally speaking, alteration of the ABA molecule showed a strong reduction of RAB-16 mRNA expression (Fig. 2A). Estimation of the RAB-16 mRNA induction obtained at 50 μM ABA was set to 100% (Fig. 2B). ABA itself, at all given concentrations, showed the best induction of RAB-16 mRNA expression. Masking of the carboxyl group by a methyl group gave about 20% reduction of the RAB-16 mRNA levels. However, when the methyl group was replaced with a glucose group, the reduction of RAB-16 mRNA expression was much more pronounced. Removal of the 1'-hydroxyl group (1'-deoxy ABA) showed 60% reduction of RAB-16 mRNA expression. When both the 1'-hydroxyl and the 4'-carbonyl groups were modified (as in α-ionylidene acetic acid), RAB-16 mRNA expression was even further reduced. Removal of all putative functional groups (α- or β-ionone) gave no RAB-16 mRNA expression at all. Table 1 shows induction of RAB-16 mRNA expression at half maximum concentration of ABA and its analogues. The results suggest an order of importance of the putative functional groups of ABA for the induction of RAB-16 mRNA: carbonyl- > hydroxyl- > carboxyl-.

Besides RAB mRNA, which is specifically regulated by ABA, there are some other mRNAs also controlled by ABA, such as BASI. The expression of BASI mRNA was studied using the ABA ana-

Table 1. Half the maximum concentration for induction of ABA-regulated gene expression by ABA analogues.

ABA analogues	RAB-16[a] (M)	BASI[b] (M)
ABA	$8 \times 10^{-7} \pm 3 \times 10^{-7}$	$5 \times 10^{-7} \pm 2 \times 10^{-7}$
ABA methyl ester	$5 \times 10^{-6} \pm 3 \times 10^{-6}$	$3 \times 10^{-6} \pm 1 \times 10^{-6}$
1'-deoxy ABA	$8 \times 10^{-5} \pm 2 \times 10^{-5}$	$2 \times 10^{-5} \pm 1 \times 10^{-5}$
ABA glucose ester	$5 \times 10^{-4} \pm 1 \times 10^{-4}$	$5 \times 10^{-4} \pm 2 \times 10^{-4}$
α-ionylidene acetic acid	$>5 \times 10^{-4} \pm 2 \times 10^{-4}$	$>5 \times 10^{-4} \pm 3 \times 10^{-4}$
α- and β-ionone	$>5 \times 10^{-3} \pm 5 \times 10^{-3}$	$>5 \times 10^{-3} \pm 5 \times 10^{-3}$

The data are the mean of four independent experiments (mean ± SD).
[a] Half the maximum concentration for induction of RAB-16 mRNA expression by ABA analogues.
[b] Half the maximum concentration for induction of BASI mRNA expression by ABA analogues.

logues (Fig. 3) to test whether ABA induced BASI expression by a similar type of interaction with a putative receptor as for RAB-16 expression. Modification of the ABA molecule effectively influenced the BASI mRNA expression as in the case of RAB-16 mRNA expression. The results for half the maximum concentration for induction of BASI mRNA expression by ABA and its analogues are also presented in Table 1. Modification of the ABA molecule gave the same order of reduction for both RAB and BASI mRNA expression.

One might argue that during the experiments some of the ABA analogues are degraded into ABA, converted to ABA due to metabolism, or affect endogenous ABA levels. In order to gain some information on possible contamination with ABA during experiments with ABA analogues, we incubated the protoplasts with different ABA analogues (final concentration 50 μM) for 2 h and the supernatant of all samples were analyzed by TLC analysis. We found that there was not trace of ABA in ABA analogues treated samples on the TLC plates. Yet the spots on TLC plates, where according to the R_f value ABA should be, were scratch off and dissolved in ethanol. The amount of ABA in all ABA-analogue-treated samples was quantified by measuring the absorbency at 240 nm. For all ABA analogues, the amount of ABA was less than 10^{-8} M, which could not induce significant RAB mRNA expression under our experimental conditions. Hence, any gene expression observed in our experiments with ABA analogues is not due to contamination with ABA during the incubation.

To make sure whether the overall transcription activity is not affected by treating the protoplasts with the ABA analogues, the expression of a gene which is not regulated by ABA was studied. We rehybridized our Northern blots with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA from barley (Cojecki 1986). With GAPDH expression in buffer set as 100%, in four independent experiments for ABA and all ABA analogues we found GAPDH expression ranging from 97–118% (SD between 8% and 12%). Thus, modification of the ABA molecule causes only a reduction of specific ABA-induced mRNA expression, but not in a gene which is not under ABA control. Although the stability of the barley GAPDH mRNA is not known, we have seen the inhibition of GAPDH expression when incubating protoplasts with Cd^{2+} (data not shown), which indicates that a reduction of the amount of GAPDH mRNA can be visualized within the given time. Furthermore, in order to ensure that the same amount of RNA was used for all samples, all our Northern blots were rehybridized with the probe of barley rRNA cDNA. Results showed that equal amounts of RNA has been loaded for Northern blots (data not shown).

Discussion

The capacity of different ABA analogues to affect ABA-induced gene expression in barley aleurone protoplasts was studied. When the carboxyl group of the ABA molecule was modified by esterification with a methyl or a glucose group, the induction of ABA-induced gene expression was partially reduced. The studies on the modification of the functional group of ABA molecule by using a monoclonal antibody (Walker-Simmons et al. 1991) and optically pure ABA analogues (Walker-Simmons et al. 1992) have been reported. In other bioassay systems—such as in the *Avena* coleoptile assay (Mcwha et al. 1973), the rapid stomatal closing assay (Uehara et al. 1975), and a rice seedling growth assay (Oritani and Yamashita 1970)—the ABA methyl ester also showed a reduced effect. Recently, Walker-Simmons et al. (1992) showed that RAB expression in wheat embryos does not have strong ABA stereochemical requirements and that other structural properties are likely important for

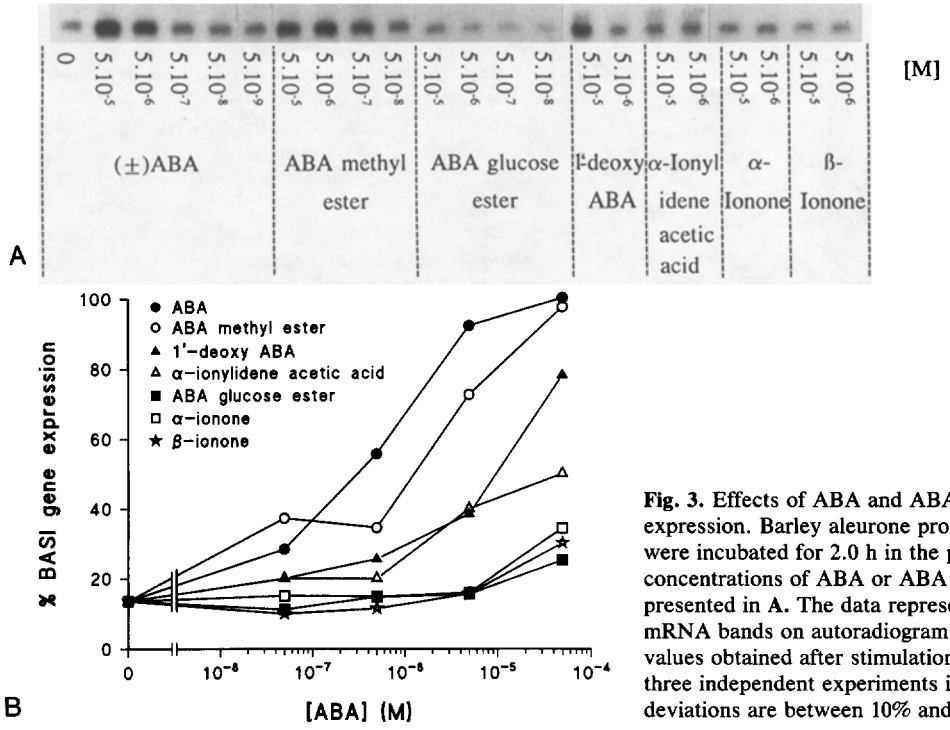

Fig. 3. Effects of ABA and ABA analogues on BASI mRNA expression. Barley aleurone protoplasts (4×10^5 protoplasts/ml) were incubated for 2.0 h in the presence of different concentrations of ABA or ABA analogues. A typical example is presented in **A**. The data represent relative OD values of BASI mRNA bands on autoradiograms expressed as percentage of values obtained after stimulation at 50 μM ABA (**B**). The mean of three independent experiments is presented. The standard deviations are between 10% and 14%.

biological activity. We tried to discover the importance of different polar groups in the ABA molecule for ABA-induced gene expression.

When the 1'-hydroxyl group of the ABA molecule was replaced with hydrogen (1'-deoxy ABA), more than 60% reduction of gene expression was found (Figs. 2 and 3). 1'-Deoxy ABA was reported to have 50% of the activity of ABA in a wheat coleoptile bioassay (Milborrow 1968). Gene expression was also sensitive to modification of the carbonyl group compared with ABA. α-Ionylidene acetic acid, which has a modification at both the 1'-hydroxyl group and the 4'-carbonyl group, showed a strong reduction of gene expression. Although α-ionylidene acetic acid was reported to be active in various bioassays (Walton 1983), it is likely that these activities were due to conversion of α-ionylidene acetic acid to ABA. In our experiments, α-ionylidene acetic acid had a very low activity in inducing gene expression.

The glucose ester of ABA was less effective in inducing RAB-16 gene expression than the methyl ester (Fig. 2). This may be explained by the fact that the glucose ester is a much larger molecule than the methyl ester, making it more difficult for the molecule to adopt a proper conformation at the binding site of the hormone receptor. Another possibility may be that the ABA methyl ester can be more easily cleaved by esterase than the ABA glucose ester (Walton 1983). As a result, the observed effects would not be attributable to the ABA ester but to ABA formed through hydrolysis. However, the absence of ABA in samples incubated for 2 h with ABA esters (TLC analysis) argues against this.

ABA analogues show the same order of reduction of both RAB-16 and BASI mRNA expression (Figs. 2 and 3), which indicates the presence of similar molecular interactions but not necessarily the identity of the receptors through which ABA induces RAB-16 and BASI mRNA expression. The specificity (Table 1) of induction of both RAB-16 and BASI mRNA expression by ABA analogues is not significantly different ($P < 0.05$). It is likely that the regulation of both the RAB and BASI gene expression by ABA occurs via the same signal transduction pathway. The specificity of ABA analogues in induction of gene expression shows the same order: ABA > ABA methyl ester > 1'-deoxy ABA > ABA glucose ester > α-ionylidene acetic acid > α- or β-ionone. Unfortunately, little is known about the location of the ABA receptor. If the ABA receptor is located on the plasma membrane (Hornberg and

Weiler 1984), ABA and all the ABA analogues do not need to pass the cell membrane. However, if the ABA receptor is located in the cytosol, the permeability of ABA analogues through the plasma membrane will strongly influence the activity of ABA analogues. It has been reported that ABA, a lipophilic weak acid, is able to diffuse into plant cells (Astle and Rubery 1980, Bianco-Colomas et al. 1991). The approximate relative polarity of ABA analogues used is the following: ABA glucose ester > ABA > 1'-deoxy ABA > α-ionylidene acetic acid > ABA methyl ester > α-ionone = β-ionone (Hansch and Leo 1979, Hansch et al. 1968, Rekker 1977). The sequence of decreasing polarity is theoretically identical to a sequence of increasing membrane permeability. The approximate relative polarity ranking of ABA analogues is completely different from the biological activity ranking of ABA analogues. ABA methyl ester is a much more lipophilic molecule than ABA. Nevertheless, ABA at all given concentrations has higher biological activity (see Figs. 2 and 3). Both α- and β-ionone are most lipophilic but have the lowest activity in inducing cellular responses. Therefore, it is very likely that the modifications of ABA molecules indicated in this paper give a reduction in activity which is not due to differences in membrane permeability of the ABA analogues.

Acknowledgments. We would like to thank Dr. B. Van Duijn for his stimulating discussion and critical reading of the manuscript. We thank John Mundy for kindly providing the RAB-16 and BASI cDNA clones. This work was supported by EUREKA grant no. EU270 and is ABIN publication no. 66.

References

Allen EF, Trewavas AJ (1987) The role of calcium in metabolic control. In: Strumpf PK, Conn EE (eds) *The biochemistry of plants 12*. Academic Press, New York, pp 117–144

Astle MC, Rubery PH (1980) A study of abscisic acid uptake by apical and proximal root segments of *Phaseolus coccineus* L. Planta 150:312–320

Bianco-Colomas J, Barthe P, Orlandini M, Le Page-Degivry MT (1991) Carrier-mediated uptake of abscisic acid by suspension cultured *Amaranthus* tricolour cells. Plant Physiol 95:990–996

Black M (1983) Abscisic acid in seed germination and dormancy. In: Addicott FT (ed) Abscisic acid. Praeger Scientific, New York, pp 331–363

Cojecki J (1986) Identification and characterization of a cDNA clone for cytosolic glyceraldehyde-3-phosphate dehydrogenase in barley. Carlsberg Res Comm 51:203–210

Dumbroff EB, Walker MA, Dumbroff PA (1983) Choice of methods for the determination of abscisic acid in plant tissue. J Chromatogr 256:439–446

Dure LS, Greenway C, Galau G (1981) Development biochemistry of cotton seed embryogenesis and germination. Changing messenger ribonucleic acid population as shown by in vitro and in vivo protein synthesis. Biochemistry 20:4162–4168

Gehring CA, Irving HR, Parish RW (1990) Effects of auxin and abscisic acid on cytosolic calcium and pH in plant cells. Proc Natl Acad Sci USA 87:9645–9649

Hansch C, Quinlan JE, Lawrence GI (1968) The linear free energy relationship between partition coefficients and the aqueous solubility of organic liquids. J Org Chem 33:347–350

Hansch C, Leo A (1979) Substituent constants for correlation analysis in chemistry and biology. J Wiley & Sons, New York

Hepler PK, Wayne RO (1985) Calcium and plant development. Annu Rev Plant Physiol 36:397–439

Hornberg C, Weiler EW (1984) High affinity binding sites for abscisic acid on the plasmalemma of *Vicia faba* guard cells. Nature 310:321–324

Leah R, Mundy J (1989) The bifunctional α-amylase/subtilisin inhibitor of barley: nucleotide sequence and patterns of seed-specific expression. Plant Mol Biol 12:673–682

Mcwha JA, Philipson JJ, Hillman JR, Wilkins MB (1973) Molecular requirements for abscisic acid activity in two bioassay systems. Planta 109:327–336

Milborrow BV (1968) Identification and measurement of (+)-abscisic acid in plants. In: Wightman F, Setterfield G (eds) Biochemistry and physiology of plant growth substances. Runge Press, Ottawa

Mundy J, Rogers JC (1986) Selective expression of a probable amylase/protease inhibitor in barley aleurone cells: comparison to the barley amylase/subtilisin inhibitor. Planta 169:51–63

Nagano E, Oritani T, Yamashita K (1980) Synthesis and physiological activity of methyl 6',6'-didemethyl-abscisate and new chiral analogs. Agri Biol Chem 44:2094–2098

Nolan RC, Ho T-HD (1988) Hormonal regulation of α-amylase expression in barley aleurone layers. Plant Physiol 88: 588–593

Oritani T, Yamashita K (1970) Studies on abscisic acid. Part 1. Synthesis of keto-ionylidene acetic acids. Agri Biol Chem 34:108–114

Rekker RF (1977) The hydrophobic fragmental constant. Elsevier, Amsterdam, The Netherlands

Rogers JC (1988) RNA complementary to α-amylase in barley. Plant Mol Biol 11:125–138

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, a laboratory manual, 2nd ed. Cold Spring Harbour Laboratory Press, Cold Spring, NY

Schubert J, Röser K, Grossmann K, Sauter H, Jung J (1991) Transpiration-inhibiting abscisic acid analogs. Plant Growth Regul 10:27–32

Skriver K, Mundy J (1990) Gene expression in response to ABA and osmotic stress. Plant Cell 2:503–512

Uehara Y, Ogawa T, Shibata K (1975) Effects of abscisic acid and its derivatives on stomatal closing. Plant Cell Physiol 16:543–546

Van der Veen R, Heimovaara-Dijkstra S, Wang M (1992) The cytosolic alkalinization mediated by abscisic acid is necessary, but not sufficient, for abscisic acid-induced gene expression in barley aleurone protoplasts. Plant Physiol 100:699–705

Walker-Simmons MK (1987) ABA levels and sensitivity in developing wheat embryo's of sprouting resistant and susceptible cultivars. Plant Physiol 84:61–66

Walker-Simmons MK, Reaney MJT, Quarrie SA, Perata P, Verieni P, Abrams SR (1991) Monoclonal antibody recognition of abscisic acid analogues. Plant Physiol 95:46–51

Walker-Simmons MK, Anderberg RJ, Rose PA, Abrams SR (1992) Optically pure abscisic acid analogs—tools for regulating germination inhibition and gene expression in wheat embryos. Plant Physiol 99:501–507

Walton DC (1983) Structure activity relationships of abscisic acid analogs and metabolites. In: Addicott, FT (ed) Abscisic acid. Praege, New York, pp 113–146

Walton DC (1987) Abscisic acid biosynthesis and metabolism. In: Davies PJ (ed) Plant hormones and their role in plant growth development. Martinus Nijhof Publishers, Dordrecht, The Netherlands, pp 113–131

Wang M, Van Duijn B, Schram AW (1991) Abscisic acid induces a cytosolic calcium decrease in barley aleurone protoplasts. FEBS Lett 278:69–74

Wang M, Van Duijn B, Van der Meulen RM, Heidekamp F (1992) Effect of abscisic acid analogues on intracellular calcium level and gene expression in barley aleurone protoplasts. In: Karssen CM, et al. (eds) Progress in plant growth regulation. Kluwer Academic Press, Dordrecht, The Netherlands, pp 635–642