Vol. 125, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1984 Pages 76-81

RAPID REDUCTION BY IAA OF MALONDIALDEHYDE LEVELS IN AVENA COLEOPTILES, A POSSIBLE EFFECT ON LIPID PEROXIDATION

Rajinder S. Dhindsa, A. Cristina Amaral and Robert E. Cleland

Department of Biology, McGill University,
Montreal, P.Q., Canada H3A 1B1 (R.S.D.)

Faculdade de Agronomia, Universidade Eduardo Mondlane,
Maputo, Mozambique (A.C.A.)

Department of Botany, University of Washington,
Seattle, WA 98195, (R.E.C.)

Received September 19, 1984

Summary: Treatment of Avena coleoptile sections with IAA results in a rapid decrease in the level of lipid peroxidation (LP), as measured by the thiobarbituric acid reaction for malondialdehyde (MDA). The response is specific for active auxins, is nearly saturated by 10^{-6} M IAA and occurs even when turgor is reduced by 0.1 M mannitol. About half the reduction in MDA occurs within 1 min after addition of IAA to intact tissues; addition of IAA directly to homogenates, however, has no effect. Homogenates prepared from auxin-pretreated tissues, but not control tissues, continue to produce MDA over at least a six-hour period. This effect of auxin on LP is one of the most rapid biochemical responses to auxin known, and suggests that LP might alter the properties of membranes and thus influence cell enlargement.

Among the most rapid effects of auxin are the promotion of proton excretion and cell elongation, both of which begin within 10 min after addition of auxin to coleoptiles (1) or stem sections (2). The possibility that auxin-induced changes in membrane lipids might be involved, either in the proton excretion or in the elongation process, has been suggested by the effects of auxin on the C18:1/C18:2 ratios of Vigna hypocotyls and stem sections (3) and by the inhibition of auxin-induced proton excretion and cell elongation of Avena coleoptile sections by the fatty acid biosynthesis inhibitor cerulenin (4). There is increasing evidence that breakdown of lipids by lipid peroxidation is directly related to several plant

Abbreviations: IAA, indole-3-acetic acid; LP, lipid peroxidation; MDA, malondialdehyde; NAA, naphthaleneacetic acid; PCIB, p-chlorophenoxyisobutyric acid; 2,4-D, 2,4-dichlorophenoxyacetic acid.

0006-291X/84 $1.50

developmental processes such as leaf senescence (5-7) and fruit ripening (8) and to membrane damage associated with seed deterioration (9) and drought stress (10).

We have tested the possibility that auxin might have a rapid effect on the lipid peroxidation of Avena coleoptiles and we demonstrate here that such a rapid response occurs.

Materials and Methods

Seedlings of Avena sativa L., c.v. Victory were grown as previously described (11). Coleoptiles, 2.5 to 3.2 cm long, were harvested and the enclosed leaf was removed. A 14 mm long section, starting from 3 mm below the tip of coleoptile, was used as experimental material.

Lipid peroxidation was measured by the thiobarbituric acid (TBA) reaction for MDA as described (12) except that the reaction mixture was heated at 80°C for 15 min as recommended (13) in order to avoid interference by sugars. Duplicate or triplicate groups of 30 coleoptile sections each were preincubated in 10 ml of 1 mM phosphate buffer, pH 6.0 for 1h before transfer to treatment solutions. Preincubation and incubation were run at 25°C in light on a rotary shaker. When mannitol was used to inhibit growth, sections were treated with 100 mM mannitol for 1h before IAA was added. At the end of incubation the coleoptile sections were homogenized in 1 ml distilled water plus 2 ml TBA reagent (12). The homogenate was heated at 80°C for 15 min in a test tube using glass marble as condenser. It was then quickly cooled in an ice-bath and centrifuged at 10 000 x g for 10 min. Supernatant was read at 535 nm and concentration of MDA was calculated (12).

When the stability of exogenously added MDA to tissue homogenates was tested the MDA was prepared from 1,1,3,3 - tetraethoxypropane as described by Brooks and Klamerth (14). Control and IAA-treated coleoptile sections were homogenized in 1 mM phosphate buffer, pH 6.0 (1ml/30 sections) and MDA was added to a final concentration of 0.1 mM. Homogenate was allowed to stand at room temperature and 0.1 ml samples in duplicate were removed at different times to measure MDA concentration.

Production of endogenous MDA by homogenates of control and IAA-treated coleoptile sections was also studied as described above except that no exogenous MDA was added.

Results

Effect of different concentrations of IAA on the MDA level of Avena coleoptile tissue is shown in Fig. 1. There is a steady decline in the level of MDA with increasing concentration of IAA until a minimum is reached at 10^{-6} M IAA.

A time-course of the effect of 10^{-5} M IAA on MDA level is shown in Fig. 2. It can be seen that a decrease of about 35% in MDA level occurs during the first 5 min of IAA application with about half this decrease occurring in the first minute (inset). A maximum decrease of about 45% is noted within 40 min of IAA application.

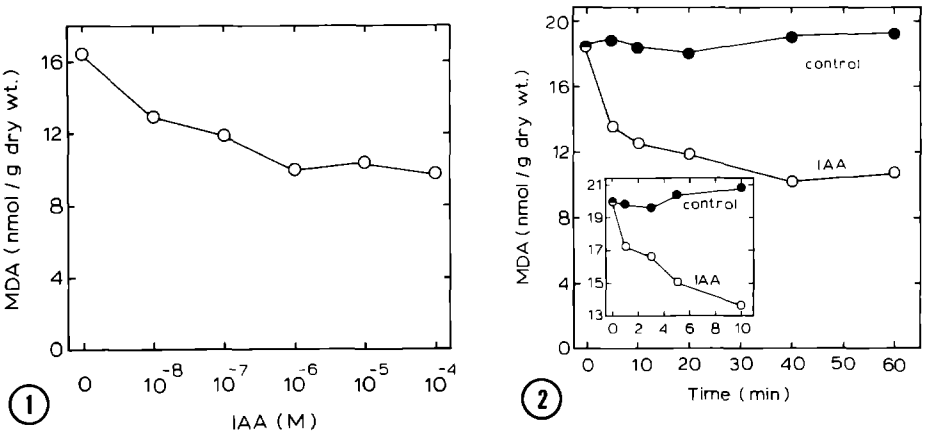

Figure 1. Reduction in MDA level by increasing concentration of IAA. Groups of 30 coleoptile sections in duplicate were preincubated in 1 mM phosphate buffer, pH 6.0, for 1 h and then treated with IAA for 40 min before being assayed for MDA level. Each value is a mean of 2 replicates. The experiment was conducted 3 times and it yielded similar results each time.

Figure 2. Time-course of reduction in MDA level by 10^{-5}M IAA. Groups of 25 coleoptile sections in duplicate were preincubated in 1 mM phosphate buffer, pH 6.0, for 1 h and then treated with 10^{-5}M IAA for different times before being assayed for MDA level. Time-course for the first 10 min is shown in the inset. Each value is a mean of 2 replicates. The experiment was conducted 3 times and it yielded similar results each time.

Several other auxins and the fungal toxin, fusicoccin, which are known to promote growth in Avena coleoptiles, have been tested for their effect on lipid peroxidation. It can be seen from Table 1 that the auxins, NAA and 2,4-D, are nearly as effective as IAA at lowering the level of lipid peroxidation in the coleoptile tissue. Fusicoccin, which is similar to auxin in its ability to induce H^+ excretion and cell elongation, on the other hand is without effect.

PCIB, an antiauxin, and benzoic acid, a weak acid with pKa close to that of IAA, are without effect on the MDA level (Table 1). Thus it appears that this reduction in LP is specific for active auxins.

The possibility that the reduction in MDA level may be a consequence of IAA-induced growth has been examined. The data in Table 1 show that the IAA-induced reduction in MDA still occurs in the presence of 0.1 M mannitol, a concentration that reduces turgor by 40% and effectively inhibits auxin-induced elongation (15).

Table 1

Treatment	MDA (nmol/g dry wt.)
Control	20.05 ± 1.84
IAA (10 μM)	12.21 ± 1.31
NAA (10 μM)	12.83 ± 1.51
2, 4-D (10 μM)	13.67 ± 1.28
Fusicoccin (10 μM)	20.75 ± 2.23
PCIB (10 μM)	18.65 ± 1.95
IAA + PCIB (both 10 μM)	16.50 ± 1.18
Benzoic acid (10 μM)	21.80 ± 1.65
Mannitol (100 mM)	19.63 ± 2.57
IAA (10 μM) + Mannitol (100 mM)	13.32 ± 2.24

Effects of auxins, fusicoccin, antiauxin (PCIB), benzoic acid and mannitol on MDA level in Avena coleoptile sections. Each value is a mean of 3 replicates ± standard error. Experiment was repeated 3 times with similar results.

The possibility that IAA might react directly with MDA has been considered. Addition of IAA directly to the TBA assay for MDA does not affect the results (data not shown). However, when homogenates were prepared from tissues pretreated with IAA for 1h, there was a continual production of MDA, until by 6h the MDA level increases to twice the initial value (Fig. 3). Such increase in MDA level does not occur when untreated control tissue is homogenized.

Discussion

Lipid peroxidation can be measured in several ways, but perhaps the most common is via the TBA assay for MDA. This has been used to assess lipid peroxidation during foliar senescence (5-7), herbicide-induced (16) and light-induced (17) damage to chloroplasts, and in microsomal membranes *in vitro* (18-20). Homogenization of Avena coleoptile tissues leads to the release of MDA, a well known product of lipid peroxidation. We show that very short treatments of the intact tissues by auxin leads to a reduction in MDA by up to 45%. There are several ways in which this reduction might occur. A direct interaction between MDA and auxin, or an interference by auxin in the MDA assay, has been eliminated. Auxin reduces the apparent

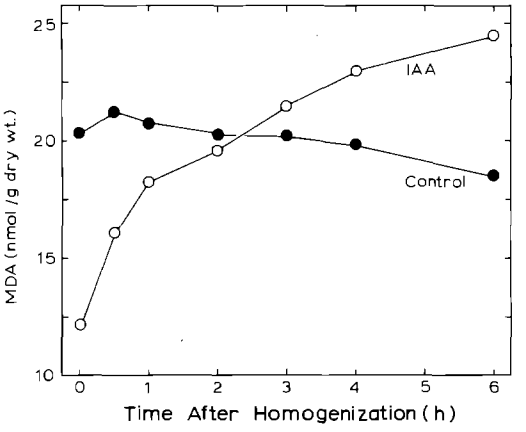

Figure 3. Time-course of endogenous production of MDA subsequent to homogenization of IAA-treated (open circles) and of untreated control (closed circles) coleoptile sections. See "Materials and Methods" for procedural details. Each value is a mean of 2 replicates. The experiment was conducted 3 times and it yielded similar results.

MDA level only when added to intact tissues, which suggests that membrane integrity is required for this response. The rapid reduction in MDA could be due to complete blockage by auxin of lipid peroxidation coupled with a rapid turnover of MDA by some process that requires intact cells. Alternatively, it could reflect an auxin-induced breakdown of MDA in vivo. None of our data would permit us to distinguish between these two possibilities.

A second effect of auxin is to potentiate the production of MDA by tissue homogenates. If tissues are not pretreated with auxin, no MDA is produced, but with a pretreatment with auxin, production of MDA by tissue homogenates continues for hours. This could mean that the membranes have been changed sufficiently by auxin so as to provide substrate for MDA production, or it could mean that the necessary enzymes are produced or activated in response to auxin.

The decrease in MDA in response to auxin is one of the most rapid biochemical effects of auxin yet discovered. Since it seems to be specific for active auxins, and occurs prior to proton excretion or enhanced cell elongation, it could be part of the sequence of events leading to growth. Decreased lipid peroxidation would change the composition of membrane

lipids and could, therefore, alter the permeability, the activity of membrane proteins, or the rate of vesicle exocytosis.

Acknowledgements

This work was supported, in part, by the Natural Sciences and Engineering Research Council of Canada grant A2724 to R.S.D.

References

1. Cleland, R.E. (1973) Proc. Nat. Acad. Sci., U.S.A. 70, 3092-3093.
2. Marrè, E., Lado, P., Caldogno, F.R. and Colombo, R. (1973) Plant Sci. Lett. 1, 179-184.
3. Dubacq, J.-P. Goldberg, R., De March, G., Prat, R., Tremolieres, A. and Lecharny, A. (1982) in: Biochemistry and Metabolism of Plant Lipids, pp. 467-470, Elsevier Biomedical Press B.V.
4. Cleland, R.E. (1983) in: Plant Growth Substances 1982, pp. 23-31, Academic Press, New York.
5. Dhindsa, R.S., Plumb-Dhindsa, P. and Thorpe, T.A. (1981) J. Exp. Bot. 32, 93-101.
6. Dhindsa, R.S., Plumb-Dhindsa, P. and Reid, D.M. (1982) Physiol. Plant 56, 453-457.
7. Venkatarayappa, T., Fletcher, R.A. and Thompson, J.E. (1984) Plant Cell Physiol. 25, 407-418.
8. Brennan, T. and Frenkel, C. (1977) Plant Physiol. 59, 411-416.
9. Stewart, R.R.C. and Bewley, J.D. (1980) Plant Physiol. 65, 245-248.
10. Dhindsa, R.S. and Matowe, W. (1981) J. Exp. Bot. 32, 79-11.
11. Cleland, R.E. (1972) Planta 104, 1-9.
12. Buege, J.A. and Aust, S.D. (1978) Meth. Enzymol. 52, 302-310.
13. Huber, C.T., Edwards, H.H. and Morrison, M. (1975) Arch. Biochem. Biophys. 168, 463-472.
14. Brooks, B.R. and Klamerth, O.L. (1968) Europ. J. Biochem. 5, 178-182.
15. Cleland, R.E. (1975) Planta 127, 233-242.
16. Van Rensen, J.J.S. (1975) Physiol. Plant. 33, 42-46.
17. Heath, R.L. and Packer, L. (1968) Arch. Biochem. Biophys. 125, 189-198.
18. Dhindsa, R.S. (1982) Phytochemistry 21, 309-313.
19. Pederson, T.C. and Aust, S.D. (1972) Biochem. Biophys. Res. Commun. 48, 789-795.
20. Shires, T.K. (1975) Arch. Biochem. Biophys. 171, 695-707.