Phytochemistry, 1973, Vol. 12, pp. 1025 to 1096. Pergamon Press. Printed in England.

ABSCISIC ACID EFFECTS ON CHROMATIN THERMAL DENATURATION

JOHN ALAN PEARSON
Department of Botany, University of Durham, Durham City

(*Received* 31 *October* 1972. *Accepted* 28 *November* 1972)

Key Word Index—*Raphanus sativus*; Cruciferae; chromatin; thermal denaturation; effect of abscisic acid.

Abstract—Extraction in the presence of 10^{-6} M abscisic acid (RS–ABA) did not give a detectable alteration in the thermal denaturation profile of radish hypocotyl chromatin. Likewise no significant changes were found when chromatin or chromatin–DNA were thermally denatured in the presence of ABA.

INTRODUCTION

INHIBITION of RNA synthesis by ABA has been demonstrated in a number of different tissues indicating a possible action of the growth regulator at the DNA template.[1–3] We have previously reported an inhibitory effect of ABA on the incorporation of ^{3}H-UMP by the chromatin-dependent RNA polymerase system from radish hypocotyls.[4] This communication reports further investigation into a possible effect of the growth regulator on the chromatin template stability as revealed by thermal denaturation determinations.

RESULTS AND DISCUSSION

Extraction of radish hypocotyl chromatin in the presence of 10^{-6} M ABA resulted in a 20–30% decrease in the incorporation of ^{3}H-UMP into TCA-insoluble material in the subsequent RNA polymerase assay.[4] One possible explanation for this effect could be that ABA changed the transcription capacity of the chromatin template; such an effect could be brought about by a change in the stability and complexing of nucleohistone. Changes of this nature have already been demonstrated for other plant growth regulators[7] where an altered T_m of nucleohistone was observed in response to pretreatment with the growth regulators. Determination of the thermal denaturation patterns of radish hypocotyl chromatin in response to ABA treatment were made to investigate a possible action at this level.

A typical thermal denaturation profile for radish hypocotyl chromatin in 0·1 SSC showed the main T_m at approximately 82° with a minor shoulder at 69·5°. This biphasic melting profile has been described by Bonner and Huang[8] and is thought to represent denaturation

[1] VILLIERS, T. A. (1968), *Planta* **82,** 342.
[2] WALTON, D. C., SOOFI, G. S. and SONDHEIMER, E. (1970), *Plant Physiol.* **45,** 37.
[3] PARANJOTHY, K. and WAREING, P. F. (1971), *Planta* **99,** 112.
[4] PEARSON, J. A. and WAREING, P. F. (1969), *Nature* **221,** 672.
[5] O'BRIEN, T. J., JARVIS, B. C., CHERRY, J. H. and HANSON, J. B. (1968), *Biochim. Biophys. Acta* **169,** 34.
[6] BONNER, J. CHALKLEY, G. R., DAHMUS, D, FAMBROUGH, D., FUJIMURA, F., HUANG, R.-C. C., HUBERMAN, J., JENSEN, R., MARUSHIGE, K., OHLENBUSCH, H., OLIVERA, B. and WIDHOLM, J. (1967), in *Methods in Enzymology* (GRASSMAN, L. and MOLDAVE, K, eds.), Vol. 12, Academic Press, New York.
[7] FELLENBERG, G. (1969), *Z. Pflanzenphysiol* **60,** 221.
[8] BONNER, J. and HUANG, R.-C. C. (1963), *J. Mol. Biol.* **6,** 169.

of the nucleohistone complex at 82° and 'uncomplexed' DNA at 69·5°. In most samples of radish hypocotyl chromatin the 69·5° stage was not seen.

The denaturation profile of control chromatin and chromatin extracted in the presence of 10^{-6} M ABA shows no differences in the T_m of the nucleohistone. The difference between the mean T_ms (control 81·1° and ABA treated 80·9) is within the experimental variation of the methods used, so it can be concluded that ABA does not appear to alter significantly the T_m of radish hypocotyl chromatin. Since the concentration of ABA used reduced ^{3}H-UMP incorporation,[4] it would appear that the growth regulator does not stabilize the chromatin and so prevent transcription of the template. T_m control 83·7; + ABA 83·9; + *cis–trans* ABA 83·15; + *trans–trans* ABA 83·2).

In further experiments, ABA ($7{\cdot}6 \times 10^{-6}$ M) was added to the mixture containing the chromatin for T_m measurement. No differences were detected between the control and treated chromatins which agrees with the lack of effect of ABA on the ^{3}H-UMP incorporation capacity of chromatin when added directly to the assay[4] (T_m control 82·0°; +ABA 83·4; +*cis–trans* ABA, 83·15; +*trans–trans* ABA 83·2).

DNA isolated from radish hypocotyl chromatin had a T_m of *ca.* 64°. Denaturation in the presence of ABA did not alter this. Kessler and Snir[9] noted that Mg^{2+} ions were required for the effect of GA_7 on the T_m of cucumber DNA, but inclusion of $MgCl_2$ at between 1 and 10 mM merely increased the T_m of the radish DNA with ABA still showing no effect.

From the above results it would appear that, although capable of reducing by 20% the incorporation of ^{3}H-UMP into radish hypocotyl chromatin, ABA caused no detectable changes in the stability of chromatin or chromatin-DNA. It seems unlikely, therefore, that the mode of action of ABA involves a direct effect on the secondary structure of the chromatin template so reducing the capacity for RNA transcription as has been proposed for other plant growth regulators.[7] The effect of ABA on RNA transcription could possibly be a secondary effect or an effect on the function of chromatin constituents other than the main template e.g. through a reduction in RNA polymerase activity.

EXPERIMENTAL

Plant materials. 4-Day-old dark-grown seedlings of radish (*Raphanus sativus* var 'Scarlet Turnip White Tip') were surface sterilized with 10 × dil. 'Chlorox' and then exhaustively washed with dist. H_2O.

Chromatin isolation. Chromatin was extracted from the excised hypcocotyls using essentially the same techniques as described.[5] Chromatin–DNA was sedimented at 100 000 g from chromatin dispersed in 4 M caesium chloride. The DNA pellet was dissolved in 0·1 SSC for thermal denaturation.

T_m *determination.* Thermal denaturation of the chromatin was performed in 0·1 standard saline citrate (SSC) in a water thermostated temperature block of a spectrophotometer. Corrections were made to compensate for non-specific light scatter.[6]

Acknowledgements—I wish to thank the University of Wales for a Postgraduate Research Studentship and I.C.I. for a Post-doctoral Research Fellowship, also Dr. R. Horgan for a gift of *cis–trans* and *trans–trans* ABA and Professor P. F. Wareing, F.R.S. for providing research facilities.

[9] KESSLER, B. and SNIR, I. (1969), *Biochim. Biophys. Acta* **95**, 207.