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EFFECTS OF ABSCISIC ACID ON NUCLEOHISTONE AND HISTONE SYNTHESIS IN MAIZE COLEOPTILES

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SUMMARY

Effects of abscisic acid (ABA) on the melting point (Tm) of nucleohistones when melted at pH 7.0 in a low ionic buffer and at pH 8.2 in a high ionic buffer were investigated.

ABA, when added in vivo, affected the Tm during the first 18 hours of ABA pretreatment neither at pH 7.0 nor at pH 8.2. Added in vitro ABA decreased the Tm of nucleohistones melted at pH 8.2 but had no effect at pH 7.0. Histone synthesis was not affected by ABA during the first 12 hours; after 24 hours a significant increase of synthesis above control was observed.

It is concluded that ABA does not affect binding between DNA and proteins in the nucleohistone complex, nor has it a direct effect on histone synthesis.

INTRODUCTION

Effects of abscisic acid (ABA) on RNA synthesis have been shown in many systems (ADDICOTT & LYON 1969). However, it is not clear by what mechanism the inhibitory effect of ABA on RNA synthesis is controlled.

Since ABA has effects on growth as well as on stomatal closure within minutes (BARKLEY & LEOPOLD 1970; CUMMINS *et al.* 1971) it seems reasonable that effects on RNA synthesis are not a direct response to ABA, but merely are a result of changes on a different level induced by ABA.

Against this are reports of a direct effect of ABA on RNA synthesis in vitro, PEARSON & WAREING (1969) reported inhibition of chromatin activity in radish hypocotyls when ABA was included in the grinding medium.

BEX (1972a) found a direct effect of ABA on the soluble RNA polymerase activity in maize coleoptiles. Inhibition of RNA synthesis could, however, not be observed during the first 2 hours of ABA treatment (BEX 1972b). There are indications that histones can inhibit the DNA dependent RNA synthesis in two different reactions: Firstly arginine-rich histones are able to bind to RNA polymerase, by which the enzyme is inactivated (SKALKA *et al.* 1966, SPELBERG & HNILICA 1969); secondly the DNA helix is stabilized by histones (WALKER 1965).

In animal systems hormones like testosteron, hydrocortison and oestradiol can bind to histones by which the binding to DNA is weakened (SLUYSER 1966a, b). FELLENBERG (1970) reported a binding of IAA to chromatin in young pea plants. Effects of IAA on the melting point (Tm) of nucleohistones in vivo and in vitro were reported (FELLENBERG 1969a); however, he melted his nucleo-

histones at pH 8.2 in a high ionic buffer. In the work reported here effects of ABA on the Tm of maize coleoptile nucleohistones in vivo and in vitro were investigated. Nucleohistones were melted at pH 7.0 in 0.015 M NaCl and at pH 8.2 in 1M NaCl.

Effects of ABA on histone synthesis were also investigated.

2. MATERIALS AND METHODS

2.1. Germination conditions and pretreatment of tissue

Air dry seeds of maize (cv. 'Kelvedon 59A') were soaked in tap water for 20 hours, transferred to vermiculite, and germinated for 5 days in the dark at 25°C. By that time the coleoptile was about 5 cm long.

The coleoptile with 1 cm of the mesocotyl was excised and used. All cuttings were performed under green safelight. For the treatments the coleoptiles with 1 cm of the mesocotyl were placed in small test tubes containing 1 ml of test solution, only the mesocotyls being immersed in the test solution.

2.2. Extraction of nucleohistones

The method used is basically that of BONNER *et al.* (1967). Three grams of tissue were homogenized in a Virtis in 10 ml of grinding medium consisting of sucrose 0.25 M, Tris/HCl buffer pH 8, 0.05 M, and $MgCl_2$ 0.001 M. The homogenate was filtered through 2 layers of cheesecloth and centrifuged at 4000 × g for 30 min.

The nucleohistone was scraped from the underlying layers and washed 5 times in 0.01 M Tris/HCl, pH 8. The final pellet was suspended in 2 ml of 1 M sucrose, layered on 15 ml 2 M sucrose in a centrifuge tube, and centrifuged at 20,000 r.p.m. in a MSE 20 ml. swing-out head. This step was repeated once. The pellet was then sheared with a Virtis and centrifuged at 2000 × g for 2 min.

2.3. Estimation of melting profile

Before the shearing the nucleohistone was dispersed in 0.01 M Tris/HCl, pH 7.0, containing 0.015 M NaCl or in 0.01 M Tris/HCl, pH 8.2, containing 1 M NaCl.

The nucleohistones were thermally denatured using a Haake constant temperature water bath pump attached to the block of a Unicam S.P. 800 spectrophotometer. The temperature was increased in 2° stages from 60°–96° allowing 10 min. after raising the temperature before recording the OD.

2.4. Extraction of histones

Two methods of histone extraction were compared. The first method is the indirect method by which histones are extracted from purified nucleohistones (BONNER *et al.* 1967). The purified nucleohistone was suspended in 1 ml 0.4M HCl and histones were extracted overnight at 4°C.

The second method is a direct and more convenient method described by STEWARD *et al.* (1971). For maize coleoptiles the procedure was slightly modi-

fied: 3 g of coleoptiles were homogenized in 5 ml. buffer containing 0.14 M NaCl in 0.01 M sodium citrate using a chilled mortar and pestle. The slurry was centrifuged at 4000 × g for 10 min, resuspended in buffer, and centrifuged again at 4000 × g; the pellet was then washed 4 times, centrifuging each time at 20,000 × g for 5 min. The final pellet was resuspended in 1 ml 0.5 M HCl and histones were extracted overnight at 4°C. The suspension was then centrifuged at 60,000 × g for 5 min. and the pellet was discarded.

Histones extracted by these two methods were electrophoretically separated on polyacrylamide gels and compared (*fig. 1*). a and b represent histones from coleoptiles extracted according to Bonner; c and d represent histones from coleoptiles extracted by the direct method of Steward; a and c are histones from untreated coleoptiles, b and d from coleoptiles treated with 3.8×10^{-5} M ABA for 24 hours.

There is no qualitative difference in the pattern of bands of all four gel strips; there is, however, a higher concentration of total histones in 30 µl of the extract made according to STEWARD *et al.* (1971).

Protein estimation of the final extracts is given in *table 1*. Part of the large amount of protein present in the HCl supernatant when extracted according to

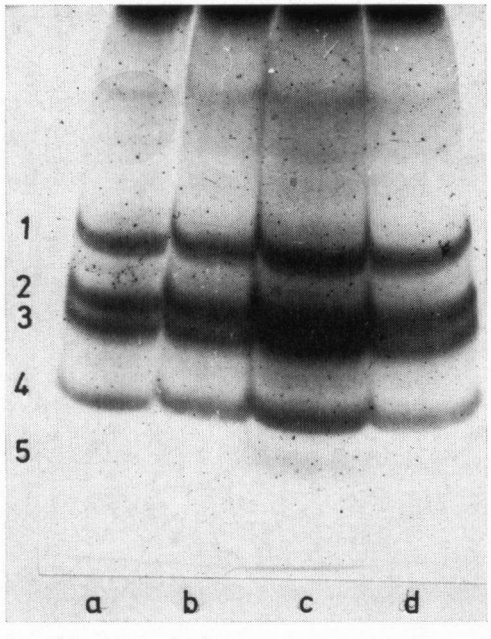

Fig. 1. Effects of ABA on histone synthesis.
a and c: control;
b and d: 3.8×10^{-5} M ABA;
a and b histones extracted according to BONNER *et al.* (1967)
c and d histones extracted according to STEWART *et al.* (1971)

Table 1. μg protein present in HCl supernatant from 1 g coleoptiles.

Extracted according to:	
Bonner *et al.*	Stewart *et al.*
140	480

Steward was probably acidic proteins. No contamination of ribosomal basic proteins could be observed.

Since there was no qualitative difference between the two methods, the direct extraction procedure for histone extraction was adapted for all further experiments.

2.5. Separation of histones using polyacrylamide gel electrophoresis

Flat slab gels (AKROYD 1968) were used for separating the histones. 10% gels were made up from 10 ml 20% acrylamide solution, 10 g of urea and 4 ml of buffer containing 31.2 g β-alanine and 8 ml acetic acid per liter (pH 4.7). As running buffer a 5 × dilution of the gel buffer was used.

30 μl of the 60,000 × g supernatant was loaded on the gel. After electrophoresis for 2 hours the slab was stained with 0.2% Coomassie Bril-liant Blue.

2.6. Estimation of ^{14}C-leucine incorporated in histones

During the last 4 hours of incubation coleoptiles were incubated in 5 μCi ^{14}C-leucine (331 mCi/mMol).

After electrophoresis the gels were stained, the histone bands cut out and counted for radio-activity in a Beckman liquid scintillation counter at 80% efficiency. Protein determination was performed according to LOWRY *et al.* (1951).

3. RESULTS

HUANG *et al.* (1964) observed a relation between the Tm and the ability to synthesize RNA on nucleohistones using *E. coli* RNA polymerase.

A low Tm was found in nucleohistones which gave a high RNA synthesis and vice versa. FELLENBERG (1969a) showed the same for different regions of the stem in young pea plants.

Since effects of ABA on Tm were investigated at pH 7.0 in a low ionic buffer and at pH 8.2 in a high ionic buffer, it was important to see whether under both conditions the same phenomena could be observed in maize plants.

Nucleohistone was extracted from coleoptiles and from roottips. The chromatin activity in the roottips should be much higher since this is a fast growing, rapidly dividing tissue in contrast to coleoptiles. The Tm of nucleohistones from roottips is lower at both pH's than the Tm from coleoptile nucleohistones (*fig. 2*). This is in agreement with what one would expect. It can also be seen that

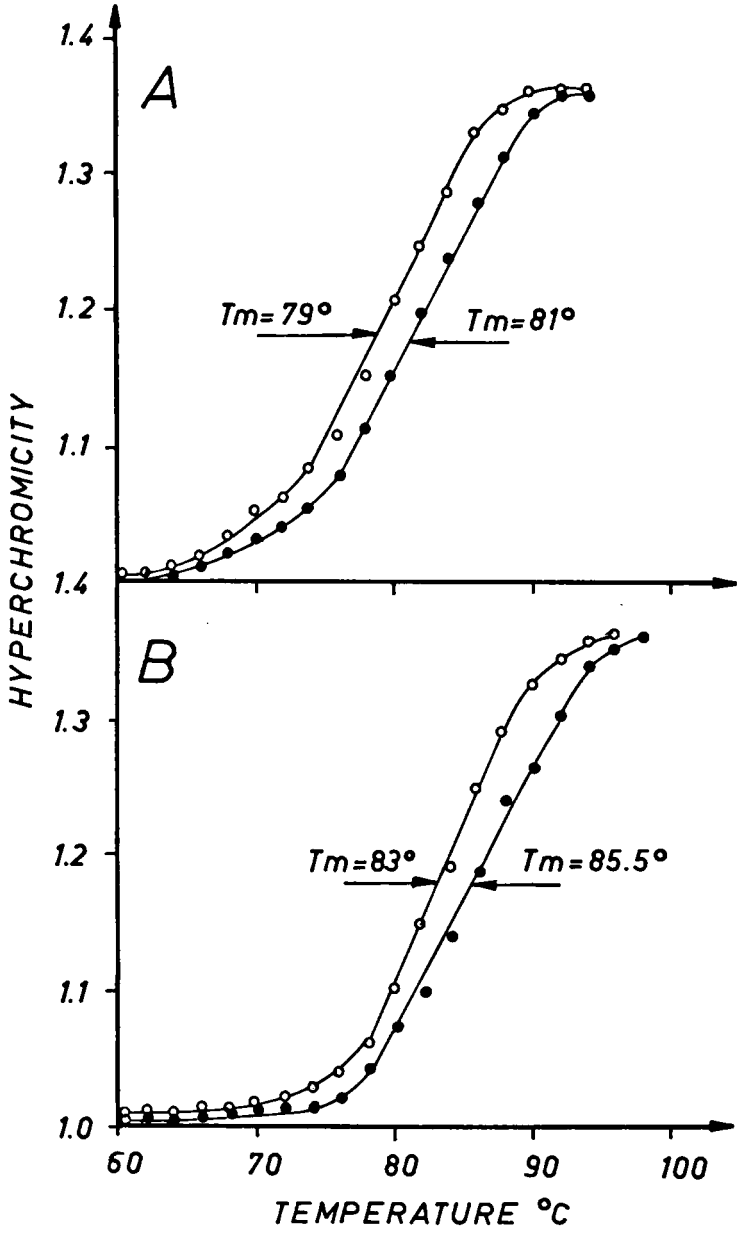

Fig. 2. Effects of different pH's on the Tm of nucleohistones extracted from roottips (A) and from coleoptiles (B).
○ Nucleohistones melted at pH 7.0 in a low ionic buffer.
● Nucleohistones melted at pH 8.2 in a high ionic buffer.

at pH 8.2 in a high ionic buffer the Tm is higher in nucleohistones from roottips as well as from coleoptiles, compared to the low ionic buffer at pH 7.0. This is in agreement with observations of CAVALIERI *et al.* (1956), who also found an increase of Tm with increasing ionic strength up to at least 1.0 M.

When coleoptiles were pretreated with 3.8×10^{-5} M ABA the Tm of the nucleohistone did not change during the first 18 hours (*fig. 3*). Only after 24 hours a small increase above the control was observed in the Tm at both pH's. The Tm of the controls also increased about 0.5° after 24 hours.

In order to investigate whether ABA binds to the nucleohistone, coleoptiles were pretreated with ^{14}C-ABA (4.7 mCi/mmol). However, after 24 hours hardly any radioactivity associated with the nucleohistone could be observed (*table 2*).

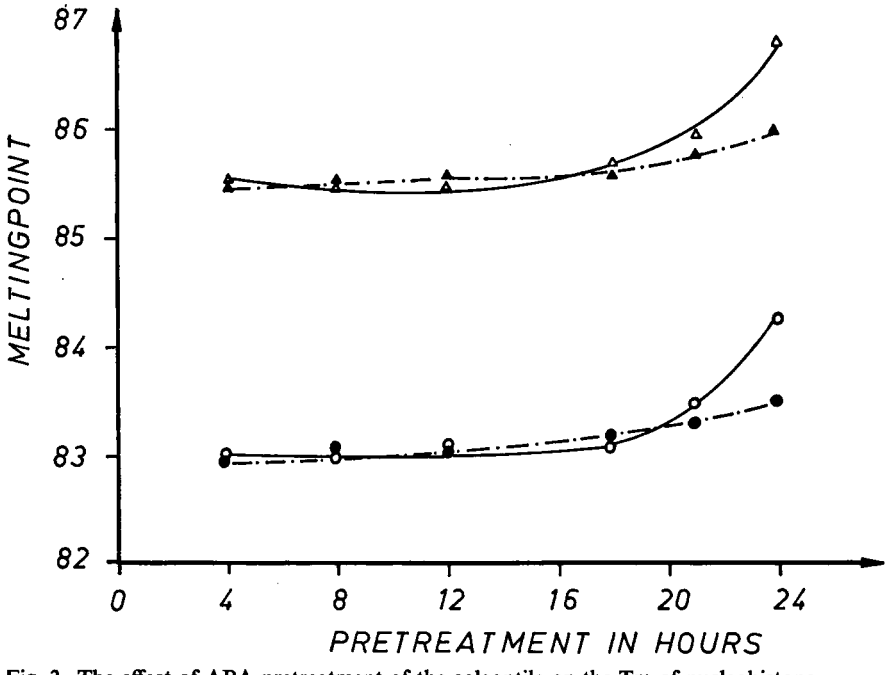

Fig. 3. The effect of ABA pretreatment of the coleoptile on the Tm of nucleohistone.
● control melted at pH 7.0
○ 3.8×10^{-5} M ABA melted at pH 7.0
▲ control melted at pH 8.2
△ 3.8×10^{-5} M ABA melted at pH 8.2

Table 2. Binding of ^{14}C-ABA to various fractions during extraction of nucleohistone from 1 g coleoptiles.

Filtered homogenate	17.400 dpm.
4000 × g pellet	4.300 dpm.
Final pellet after 5 × washing	< 300 dpm.
After sucrose dens. centrif.	< 50 dpm.

Table 3. Effects of ABA on the Tm of nucleohistone in vitro.

Conc. of ABA in M.	Tm at pH 7.0	Tm at pH 8.2
0	81°	85.5°
10^{-8}	81°	85.5°
10^{-7}	81°	85°
5×10^{-7}	81°	84°
10^{-6}	81°	82.5°

This does not, however, imply that ABA does not interact with nucleohistones, since during the extraction of the nucleoprotein the ABA may have been removed from the nucleohistones.

To investigate the effect of ABA on the nucleohistone in vitro, nucleohistones were extracted from maize coleoptiles and treated with different concentrations of ABA for one hour at room temperature.

When the nucleohistone was melted at pH 8.2 in 1M NaCl, the addition of ABA to the nucleohistone resulted in a decrease of the Tm (*table 3*). When melted at pH 7.0, however, no difference in the Tm could be observed at any concentration of ABA.

Since small changes in nucleohistones are not detectable with the relatively gross method of Tm estimation, effects of ABA on histone synthesis were investigated. A pre-incubation of coleoptiles in 3.8×10^{-5} M ABA for 6 or 12 hours had no effect on histone synthesis (*table 4*). Very little histone synthesis

Table 4. Effects of 3.8×10^{-5} M ABA on the histone synthesis in maize coleoptiles.

Time of ABA treatment in hours	Histone fraction	dpm/g. fr. wt. in control	dpm/g. fr. wt. in ABA treated	% increase over control
	1	290	310	7
	2	390	380	–3
6	3	180	200	11
	4	230	210	–9
	5	450	470	4
	1	380	380	0
	2	490	470	–4
12	3	240	200	–17
	4	290	340	17
	5	530	500	–6
	1	760	1020	34
	2	1030	1390	35
24	3	490	660	35
	4	660	920	39
	5	1090	1440	32

could be observed in the treated and untreated tissue. After 24 hours an increase of histone synthesis was observed in the ABA treated coleoptiles. In the control coleoptiles the histone synthesis also increased but to a lesser extent. However, when the increase of histone synthesis in the ABA treated coleoptile was expressed as a percentage of the control, very little difference could be found between different histone fractions.

4. DISCUSSION

A decrease of the soluble RNA polymerase activity has been reported when ABA was included in the grinding medium (BEX 1972b). In vivo experiments showed, however, that RNA synthesis in maize coleoptiles was inhibited after 3 hours of ABA treatment (BEX 1972a), but soluble RNA polymerase activity only decreased after 6 hours (BEX 1972b). This indicates that before ABA affects the RNA polymerase activity other factors controlling RNA synthesis have changed.

Hormones affect the binding of proteins to DNA in animal systems (SLUYSER 1966a and b, MAURER & CHALKLEY 1967), as well as in plant systems (FELLENBERG 1969a and b). The fact that ABA in vivo has only an effect on the Tm after 24 hours pretreatment of the coleoptiles, and that in vitro only at pH 8.2 in a high ionic buffer the Tm of the nucleohistone complex could be influenced, indicates that ABA not directly influences the binding of histones to DNA. Addition of ABA to the nucleohistone at pH 8.2 lowered the Tm; this would thus correspond to a higher level of RNA synthesis according to the correlation established by HUANG *et al.* (1964). In vivo, however, ABA lowered RNA synthesis (BEX 1972a). This means that the effect of ABA on the nucleohistone complex in vitro at pH 8.2 is an artefact, especially since at both pH's a true difference in chromatin activity also results in a difference in Tm (*fig. 2*). FELLENBERG (1969a and b) observed in vitro an effect of IAA, kinetin and GA on the Tm of nucleohistones from pea epicotyls. However, he always 'bound' his growth substances at a pH between 8.0 and 8.5 in a high ionic buffer. Thus our observations make it necessary to reconsider the physiological significance of Fellenberg's results.

Very little histone synthesis was observed during the first 12 hours of incubation of coleoptiles in ABA (*table 3*). The slight increase in histone synthesis in control tissue after 24 hours is probably the cause of the increase of Tm after the same period of incubation in water (*fig. 3*). These changes may be a result of a lack of reserve materials in the detached coleoptiles which also inhibits growth of the coleoptile compared to coleoptiles attached to the whole plant (Bex, unpublished results).

The stimulation of histone synthesis after 24 hours ABA treatment is probably a result of inhibition of growth by ABA which means that it is the same response to the growth inhibition as that observed in the control tissue, only in a stronger way.

The results showed that ABA did not affect the binding of DNA and proteins

in the nucleohistone complex, nor did it directly stimulate histone synthesis.

Effects of ABA on nucleohistone in vitro at pH 8.2 most probably are an artefact since ABA did not affect the Tm at pH 7.0.

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