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# THE EFFECT OF PLANT GROWTH REGULATORS ON DNA

ELCHANAN S. BAMBERGER

Department of Biochemistry, Tel-Aviv University, Tel-Aviv, Israel

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**Abstract**—Purified DNA from pea seedlings was incubated at low ionic strength with each of the following plant growth regulators: Indole-3-acetic acid (IAA); Gibberellic acid (GA); 2,4-Dichlorophenoxy acetic acid (2,4-D); 2,4,5-Trichlorophenoxy acetic acid (2,4,5-T); Indole-3-propionic acid (IPA); Indole-3-butyric acid (IBA); 6-Benzylaminopurine (BAP) and 6-Furfurylaminopurine (KIN). Alterations in the thermal denaturation transition profiles and the melting temperature (Tm) were used to evaluate the effect of these substances on the conformation of the treated DNA. At concentrations of $10^{-4}$ M to $10^{-3}$ M, IAA, GA, 2,4-D and 2,4,5-T caused a decrease of the Tm. BAP and KIN at a concentration of $10^{-4}$ M prevented the hyperchromicity resulting from denaturation of DNA. At $10^{-4}$ M, IBA and IPA caused an upward shift of the Tm, but at higher concentrations both compounds caused a downward shift of the Tm.

## INTRODUCTION

WHILE the exact mode of the primary action of plant hormones is not yet known, it now appears that plant hormones, as well as animal hormones are involved in gene activation and repression.[1-5] Hormones are likely to operate on more than one level in the cell, but attention has mostly been focused on the effects of hormones on the transcription and translocation processes as well as on the protein synthesizing machinery.[1,6] Autoradiographic evidence revealed the localization of administered testosterone[1] and aldosterone[7] within the chromosomes. Microscopic observation showed that specific areas of certain chromosomes may be puffed by ecdysone and that puffing is associated with increased m-RNA synthesis.[8] Chromosomal puffing might be a magnification of the minute, but additive alterations in the physical status of the hormone-activated DNA molecules comprising the chromosome. This latter hypothesis was tested by Goldberg *et al.*[9] who observed that incubation of DNA from human placental nuclei with a variety of hormones brought about a distinct change in the DNA melting profile. These results are an indication that one possible action of hormones in gene activation occurs by promoting the separation of complementary strands of specific segments of the DNA double helix prior to transcription. Recently, Cohen and Kidson[10] reported that hormonal steroids under equilibrium conditions bind to denatured but not to native DNA. Fellenberg[11,12] reported recently that IAA,

[1] E. H. DAVIDSON, *Sci. Am.* **212,** 36 (1965).
[2] J. V. JACOBSEN and J. E. VARNER, *Plant Physiol.* **42,** 1596 (1967).
[3] M. M. JOHRI and J. E. VARNER, *Proc. Natl. Acad. Sci. U.S.* **59,** 269 (1968).
[4] L. D. NOODEN and K. V. THIMANN, *Proc. Natl. Acad. Sci. U.S.* **50,** 194 (1963).
[5] P. O. P. Ts'O, G. K. HELMKAMP and C. SANDER, *Proc. Nat. Acad. Sci. U.S.* **48,** 686 (1962).
[6] J. R. TATA, *Nucleic Acid Research and Molecular Biology* (edited by J. N. DAVIDSON and W. E. COHN), Vol. 5, pp. 191–250, Academic Press, New York (1966).
[7] P. M. LOEB and J. D. WILSON, *Clin. Res.* **13,** 45 (1965).
[8] U. CLEVER, in *The Nucleohistones* (edited by J. BONNER and P. Ts'o) p. 317, Haldenday, San Francisco, (1964).
[9] M. L. GOLDBERG and W. A. ATCHLEY, *Proc. Natl. Acad. Sci. U.S.* **55(a)** 989 (1966).
[10] P. COEHN and C. KIDSON, *Biochem.* **63,** 458 (1969).
[11] G. FELLENBERG, *Planta Berl.* **84,** 324 (1969).
[12] G. FELLENBERG, *Z. Pflanzenphysiol.* **60,** 457 (1969).

GA and KIN interact *in vitro* with nucleoproteins and deproteinized DNA of root forming pea epicotyls and affect their Tm. The Tm of the nucleoproteins was decreased by these hormones, while the Tm of the deproteinized DNA was increased by KIN but decreased by GA and IAA. Kessler and Snir[13] showed that the plant hormone gibberellin $A_7$ does specifically change the physical state of DNA, resulting in changes in the thermal denaturation profiles of DNA-gibberellin $A_7$ complexes.

It is the purpose of the present paper to show that a number of plant hormones and some plant growth regulators interact with purified DNA and affect its melting profile. A preliminary report of this work has appeared elsewhere.[14]

## RESULTS

The experimental results are shown in Figs. 1–8. In the melting profile figures the ordinate shows the difference ($\Delta OD_{260}$) between the absorbancy, $A_t$, at the indicated temperature and the absorbancy $A_o$, at room temp. The data summarized in any one figure were obtained in the same experiment. Curves obtained in replicate experiments were similar but not identical, probably due to differences in the DNA preparations, and unavoidable variation in the rate of heating.

### *Dependence of the Tm on the Ionic Strength*

The stability of DNA to denaturation usually increases with increasing ionic strength of the solvent.[15,16] In order to isolate the effect of the growth regulator on the Tm from the ionic strength effect, the Tm was determined at the lowest salt concentration which was just enough to prevent denaturation of DNA before the start of the experiment. Figure 1

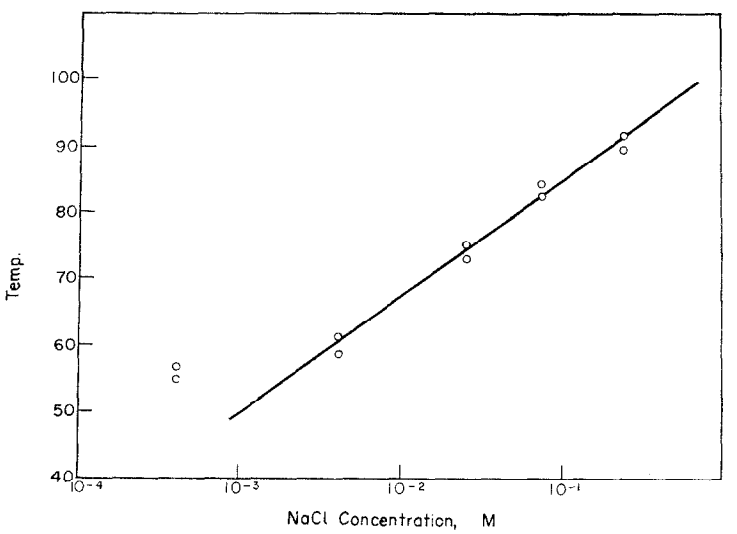


FIG. 1. EFFECT OF THE IONIC STRENGTH ON THE Tm OF DNA FROM PEA SEEDLINGS. 25 μg/ml DNA WERE SUSPENDED IN VARIOUS CONCENTRATIONS OF SSC ADJUSTED TO pH 7·0. THE EXPERIMENT WAS CARRIED OUT AS DESCRIBED IN EXPERIMENTAL.

[13] B. KESSLER and I. SNIR, *Biochim. Biophys. Acta* **195**, 207 (1969).
[14] E. S. BAMBERGER and M. SHARABANI, *Israel. J. Chem.* **7**, 122 p. (1969).
[15] W. F. DOVE and N. DAVIDSON, *J. Mol. Biol.* **5**, 467 (1962).
[16] J. MARMUR and P. DOTY, *J. Mol. Biol.* **5**, 109 (1962).

depicts the increase of the Tm of pea seedling DNA as a function of salt concentration, from $3 \times 10^{-4}$ M to $1{\cdot}5 \times 10^{-1}$ M NaCl. The slope of the curve increases by $\sim 17{\cdot}0°/\log [Na^+]$. Sodium chloride at $3 \times 10^{-3}$ M (1/50 SSC) was chosen as the salt concentration for the determination of the effect of the growth regulators on the Tm. It was calculated, by means of the u.v. spectral analysis for nucleic acids of Hirschman and Felsenfeld,[17] that at $3 \times 10^{-3}$ M NaCl about 60% of the DNA was native at the start of the Tm measurement. The concentration of nucleotides which were denatured was between $2 \times 10^{-5}$ M to $5 \times 10^{-5}$ M.

*The Effect of the Growth Regulators on the Tm*

Preliminary tests showed that no change in absorbancy resulted from the heating of IAA, tryptophan (TRY) 2,4-D or 2,4,5-T in solutions without added DNA. It was therefore possible to record the spectra of these growth regulators plus DNA solutions against a reference of the appropriate concentration of growth regulator in SSC buffer. However, since GA by itself showed a concentration dependent hyperchromic effect, in experiments with GA the thermal transition curves obtained for DNA plus GA solutions were corrected for the temperature dependent increase in absorbancy of GA itself. Similarly, the absorbancies of KIN and BAP alone varied with increasing temperature and corrections were made accordingly.

*IAA.* The effect of IAA on the thermal transition of DNA is shown in Fig. 2. There was no detectable effect up to a concentration of $10^{-4}$ M. The Tm decreased with increasing IAA concentrations in the medium, and reached a value of 49·6° at $2{\cdot}5 \times 10^{-4}$ M IAA, as compared with the control value of 64·4°. Tryptophan, which is structurally related to IAA, had no effect on the Tm when it was present in the same molar concentration as IAA (insert to Fig. 2).

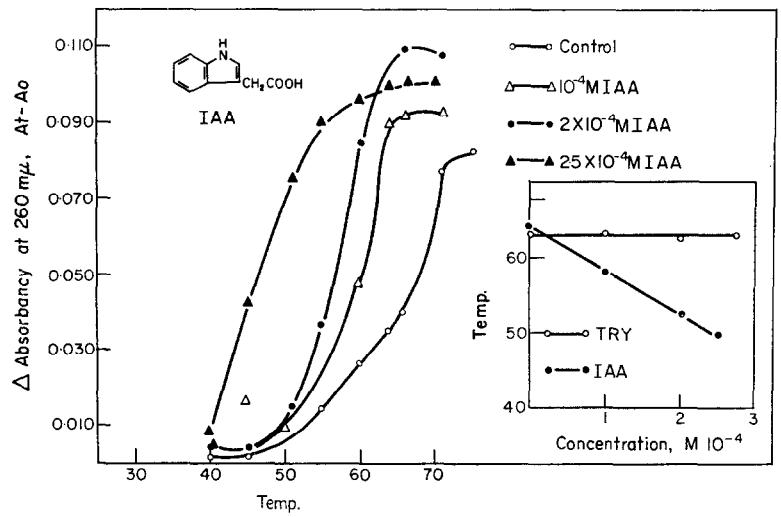


FIG. 2. THERMAL DENATURATION PROFILES OF DNA (25 μg/ml) FROM PEA SEEDLINGS IN THE PRESENCE OF VARYING IAA CONCENTRATIONS IN 1/50 SSC pH = 7·0.
In the insert: the Tm's obtained from the experiments with IAA and similar experiments with TRY are plotted.

[17] S. Z. HIRSCHMAN and G. FELSENFELD, *J. Mol. Biol.* **16**, 347 (1966).

*IPA and IBA.* Indole propionic acid and indole butyric acid are generally less active than IAA as growth promoting substances due to their longer side chain; two carbons plus an oxygen on the side chain of the indole ring appear to be optimal for auxin activity.[18] Both IPA and IBA effected the thermal transition profile of DNA in a different manner than did IAA. IPA (Fig. 3) caused a slight upward shift of the Tm at $10^{-4}$ M, and a downward shift at $3 \times 10^{-4}$ M. At a lower salt concentration ($3 \times 10^{-4}$ M NaCl) the biphasic and opposite response to IPA was somewhat more pronounced. As shown in Fig. 3 the effect of IBA on DNA was similar to the effect of IPA. Again at the lower salt concentration ($3 \times 10^{-4}$ M NaCl) a biphasic response is apparent.

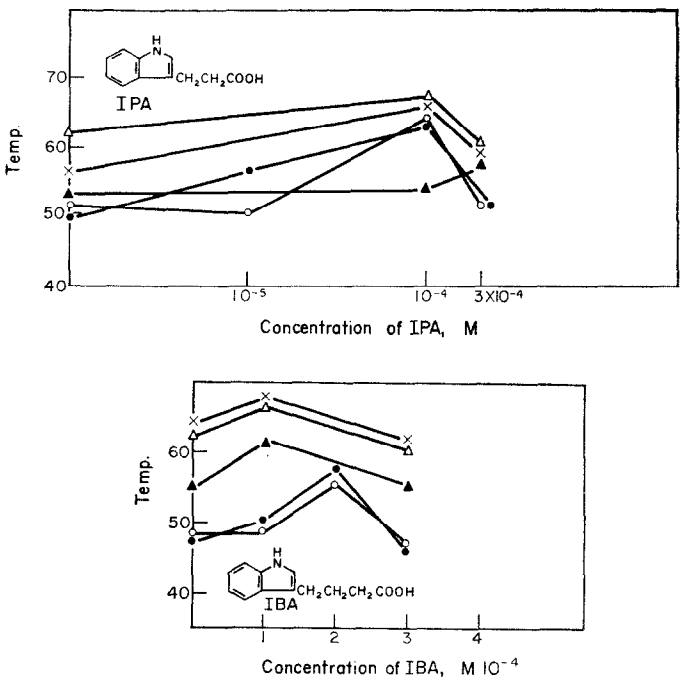


FIG. 3. DEPENDENCE OF THE Tm OF DNA (25 μg/ml) FROM PEA SEEDLINGS ON VARYING CONCENTRATIONS OF IPA (TOP) AND IBA (BOTTOM) AT DIFFERENT IONIC STRENGTH: □—□, △—△, ▲—▲ AT 1/50 SSC pH = 7·0; ○—○, ●—●, AT 1/500 SSC pH = 7·0.

*2,4-D, 2,4,5-T and GA.* In Figs. 4–6 the thermal transition profiles of DNA in the presence of increasing concentrations of 2,4-D, 2,4,5-T and GA are drawn respectively. All three growth regulators caused a concentration dependent downward shift of the Tm. Even though all three compounds cause a labilization of the DNA, the extent of this effect is different with each one of them. This can be seen from the different shapes of the transition profiles as well as from the slope of the plots of the Tm against growth regulator concentration (see inserts in Figs. 4–6).

*KIN, BAP.* The effect of the cytokinins KIN and BAP on the denaturation of DNA is depicted in Fig. 7. KIN, at $10^{-4}$ M, abolished the hyperchromicity effect. Benzylaminopurine

[18] J. B. KOEPFLI, K. V. THIMAN and F. W. WENT, *J. Biol. Chem.* **122**, 763 (1938).

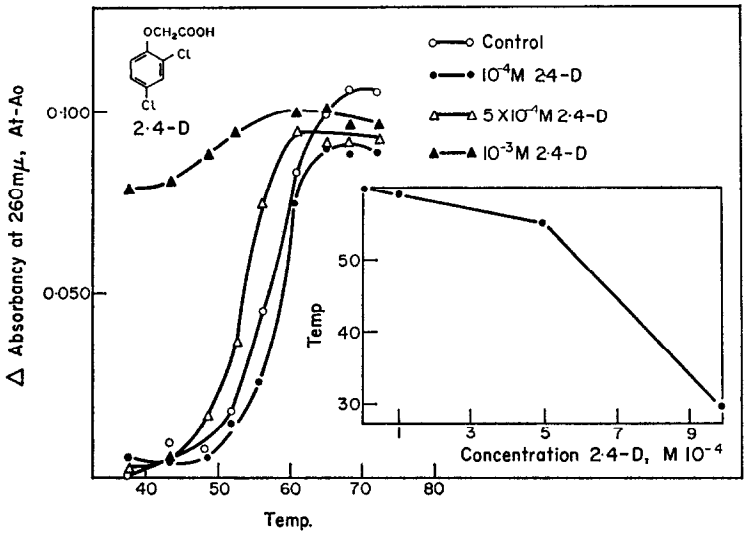


FIG. 4. THERMAL DENATURATION PROFILES OF DNA (25 μg/ml) FROM PEA SEEDLINGS IN THE PRESENCE OF VARYING CONCENTRATIONS OF 2,4-D IN 1/50 SSC pH = 7·0.
In the insert: the Tm's obtained are plotted against 2,4-D concentration.

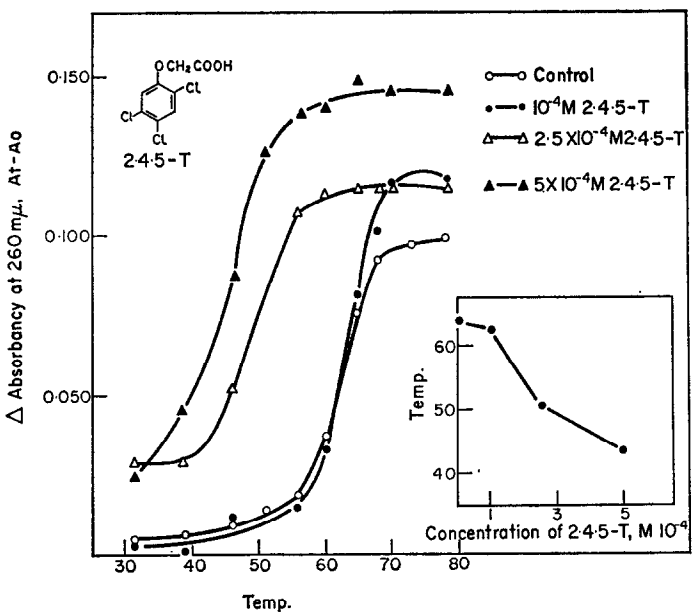


FIG. 5. THERMAL DENATURATION PROFILES OF DNA (25 μg/ml) FROM PEA SEEDLINGS IN THE PRESENCE OF VARYING CONCENTRATIONS OF 2,4,5-T IN 1/50 SSC pH = 7·0.
In the insert: the Tm's obtained are plotted against 2,4,5-T concentration.

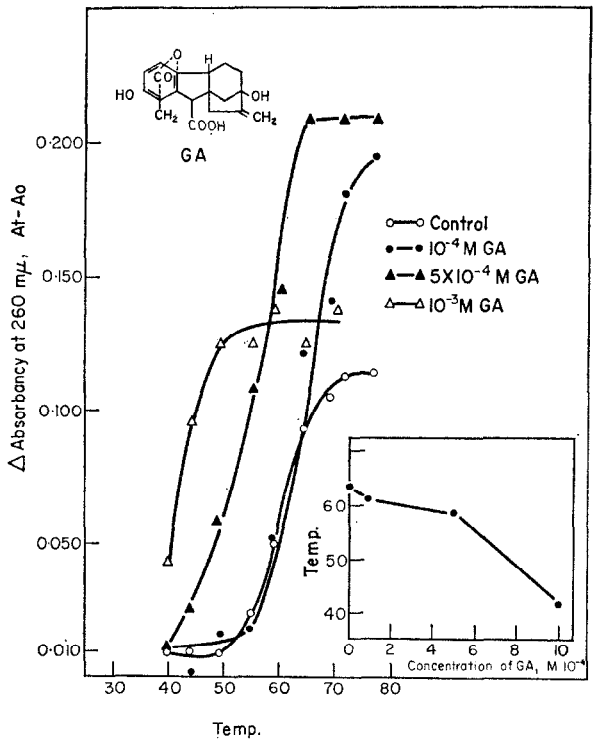


FIG. 6. THERMAL DENATURATION PROFILES OF DNA (25 μg/ml) FROM PEA SEEDLINGS IN THE PRESENCE OF VARYING CONCENTRATIONS OF GA IN 1/50 SSC pH = 7·0.
In the insert: the Tm's obtained are plotted against GA concentration.

had the same effect at $7{\cdot}5 \times 10^{-5}$ M. However, when the salt concentration in the medium was increased to $3 \times 10^{-2}$ M NaCl, KIN and BAP caused only a small upward shift of the Tm but the extent of the hyperchromicity was reduced.

*The Effect of Plant Growth Regulators on Calf Thymus DNA*

It was interesting to know whether plant growth regulators effect just plant DNA or DNA from any source. The effect of 2,4-D, 2,4,5-T and GA on the Tm of highly polymerized calf thymus DNA (Worthington) at $3 \times 10^{-3}$ M NaCl is shown in Fig. 8. In general, these compounds had the same effect on calf thymus DNA as on pea seedlings DNA, namely, they caused a decrease in the Tm.

## DISCUSSION

In the experiments described in this paper, we examined the effect of a number of plant hormones and plant growth regulators on the thermal stability of DNA isolated from pea seedlings. Three types of interactions with DNA emerge from these studies: (a) The plant hormones IAA and GA and the synthetic growth regulators 2,4-D and 2,4,5-T destabilized portions of the native DNA molecules as reflected by the downshift of the Tm (Figs. 2, 4–6). (b) The natural growth factor KIN and the synthetic cytokinin BAP, at low ionic strength,

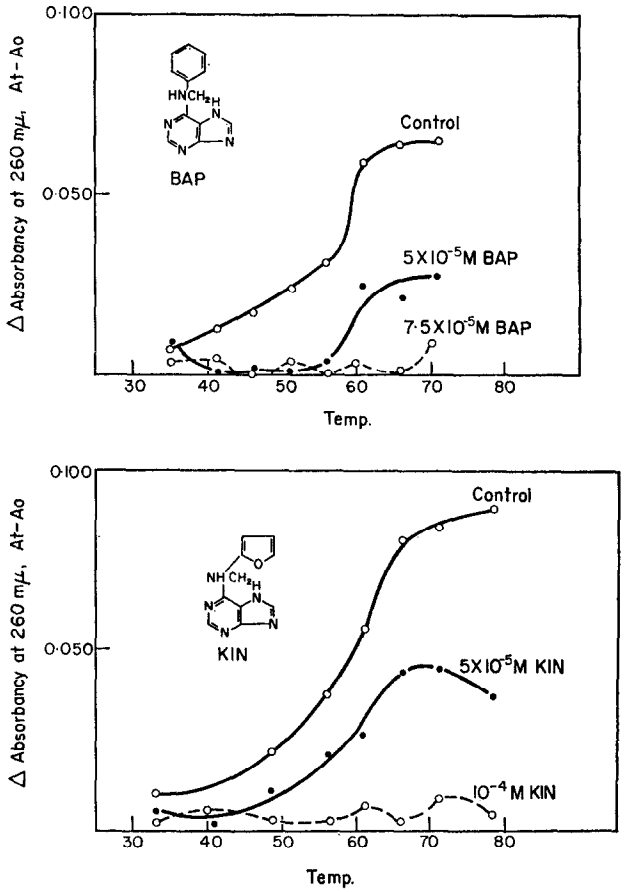


FIG. 7. THERMAL DENATURATION PROFILES OF DNA (25 μg/ml) FROM PEA SEEDLINGS IN THE PRESENCE OF VARYING CONCENTRATIONS OF BAP (TOP) AND KIN (BOTTOM) IN 1/50 SSC pH = 7·0.

stabilized DNA to the extent that at a concentration of $10^{-4}$ M the hyperchromicity of DNA was completely abolished (Fig. 7). (c) Native DNA showed a biphasic and opposite response to increasing concentrations of IBA and IPA. Low concentrations ($10^{-4}$ M) of IPA and IBA led to stabilization, and higher concentrations ($3 \times 10^{-4}$ M), to labilization of the native structure of DNA (Fig. 3). The results with IAA, GA and KIN are similar to those obtained by Fellenberg.[11,12]

The question that comes to mind is what hypothesis can account for these kinds of stabilization and labilization effects? The simplest hypothesis is that which postulates that destabilization is brought about by binding of the non-polar structures of the growth regulators to certain sites on the DNA helix. At these sites, they act as denaturants, probably by virtue of stronger affinity for the polynucleotide in its disordered, rather than its ordered conformation. Stabilization, however, is possibly caused by preferential formation of a stronger complex between the organic ligand of the growth regulator and the native helical conformation, compared to the denatured coil conformation. This hypothesis is in accord

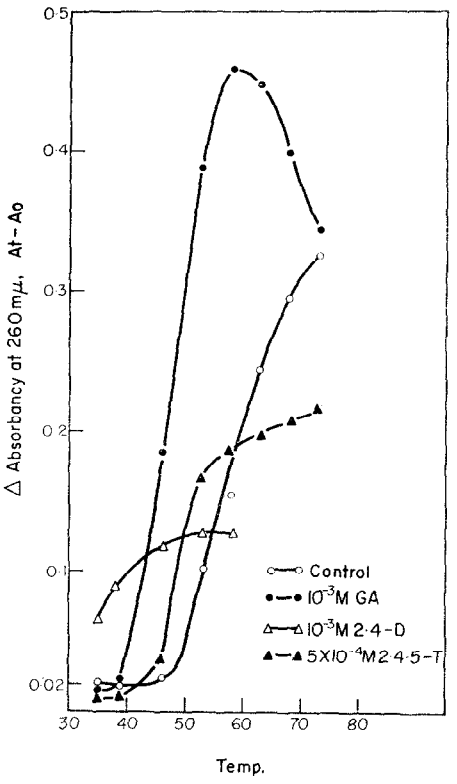


FIG. 8. THERMAL DENATURATION PROFILES OF HIGHLY POLYMERIZED CALF THYMUS DNA (25 μg/ml) IN THE PRESENCE OF $10^{-3}$ M GA, $10^{-3}$ M 2,4-D AND $5 \times 10^{-4}$ M 2,4,5-T IN 1/50 SSC pH 7·0.

with the helix coil transition theory and the mechanism usually proposed for stabilization and destabilization of helical polymers.[5,15,19]

A number of organic substances have been shown to affect the m.p. of DNA, at concentrations similar to those used in this study ($10^{-5}$ M–$10^{-3}$ M). The effect was generally nonspecific.[20] Antibiotics, such as actinomycin D at concentrations between 5 and 25 μM, form stable complexes with DNA, inhibit DNA dependent RNA polymerase, and stabilize native DNA to thermal denaturation.[21] Goldberg and Atchley[9] showed reproducible alterations in the melting profile of DNA in the presence of representatives of three groups of hormones: steroid, protein and amino acid derivatives at concentrations similar to those found in the intact organism. These hormones showed an extraordinary degree of specificity.

The concentrations of plant hormones and growth regulators used in this study which affect the stability of DNA, are within the upper range of the concentrations generally used in physiological studies.[22] Ten to fifty molecules of hormone of growth regulator per one nucleotide in the native DNA are required to obtain maximum activity of these compounds ($5 \times 10^{-4}$ M–$10^{-3}$ M growth regulator per $5 \times 10^{-5}$ M nucleotides). In the case of the steroid

[19] S. LIFSON and B. H. ZIMM, *Biopolymers* **1**, 15 (1963).
[20] L. LEVINE, L. A. GORDON and W. P. JENCKS, *Biochem.* **2**, 168 (1963).
[21] W. KERSTEN, H. KERSTEN and W. SZYBALSKI, *Biochem.* **5**, 236 (1966).
[22] A. W. GALSTON and P. J. DAVIES, *Science* **163**, 1288 (1969).

hormones,[9] the ratio of hormone to nucleotides is in the order of 1:1. Protein hormones, however, were effective at much lower concentrations, insulin at $6{\cdot}9 \times 10^{-10}$ M and somatotropin at $10^{-13}$ M. It is clear that in addition to the chemical nature of the hormone molecule, its size is an important factor in determining its effect on the stability of DNA. This might explain the requirement for relative high concentrations of the small molecules of plant growth regulators to achieve a measureable alteration of the thermal denaturation curve of DNA. One has to bear in mind that with the thermal denaturation method, drastic changes of the DNA molecule are measured; at the Tm, 50% of the DNA molecules are denatured. *In vivo*, hormones or growth regulators probably denature or stabilize only very small portions of DNA molecules. Presumably, only a few genes are turned on or off by a given hormone. Therefore, the effects of physiological concentrations (e.g. $10^{-8}$ M–$10^{-7}$ M for GA) of rather small molecules, do not influence the melting temperature curve. However, the shifts in the Tm by higher concentrations as reported in this paper indicate the potential of these compounds and the role they can assume at the level of DNA in the control of nucleic acids and protein synthesis. Based on similar observations Fellenberg[11,12] postulated that IAA and GA are 'true initiators' of RNA synthesis since they loosen both the binding of histone to DNA and DNA to DNA in the double helix.

Indeed, in many plant tissues, an increase of protein and RNA synthesis is evoked by hormones and growth regulators.[4,23–25] Gibberellic acid, for example, promotes a *de novo* synthesis of α-amylase and enhances the synthesis of ribonuclease[26] and protease[2] in germinating barley grains. In isolated pea nuclei gibberellic acid preferentially enhanced the incorporation of tritiated cytidine triphosphate into RNA associated with DNA, but had no effect on ribosomal RNA.[3] The destabilization of DNA by GA (Fig. 6) fits in well with the idea[3] that GA increases the number of template sites on DNA, followed by a synthesis of a small but specific RNA fraction.

The specificity of the effect of plant hormones and regulators on the melting properties of plant DNA is obscure at this stage. We have demonstrated that tryptophan, which is a structural analogue of IAA, IPA and IBA, and has no hormonal activity, had no effect on the Tm (Fig. 2). However, IBA and IPA (Fig. 3) which are generally less active auxins than IAA[18] affected DNA in a different manner than IAA. In addition, GA, 2,4-D and 2,4,5-T destabilize calf thymus DNA (Fig. 8). It is, however, reasonable to postulate that specificity *in vivo* results from a complex of DNA, the hormone, and the 'reading head' of the DNA dependent RNA polymerase. The nature of this complex, the binding of plant hormones and growth regulators to DNA or their efiect on the activity of DNA dependent RNA polymerase remains to be determined.

## EXPERIMENTAL

### *Reagents*

All chemicals were of reagent grade. Only glass distilled $H_2O$ was used. As a general rule, all substances were dissolved in the appropriate dilution of standard saline citrate designated SSC (1 × SSC = 0·15 M sodium chloride, 0·015 M sodium citrate adjusted to pH = 7·0).

### *Hormones*

2,4-Dichlorophenoxy acetic acid (2,4-D), 2,4,5-Trichlotophenoxy acetic acid (2,4,5-T), 6-furfurylaminopurine (KIN) and Tryptophan (TRY) were obtained from Mann Research Laboratories. 3-Indole acetic

[23] F. B. Abeles and J. E. Holm, *Plant Physiol.* **41**, 1337 (1966).
[24] J. L. Key and J. Shannon, *Plant Physiol.* **39**, 360 (1964).
[25] J. E. Varner and G. R. Chandra, *Proc. Natl. Acad. Sci. U.S.* **52**, 100 (1964).
[26] M. J. Chrispeels and J. E. Varner, *Plant Physiol.* **42**, 398 (1967).

acid (IAA) and 6-Benzylaminopurine (BAP) were obtained from Sigma. 3-Indole propionic acid (IPA), and 3-Indole butyric acid (IBA) from Eastman Organic Chemicals; and Gibberellic acid (GA) from Nutritional Biochemicals Corporations.

*Preparation of Plant DNA*

DNA was prepared from 6-day-old etiolated pea stems (*Pisum sativum* var. *Alaska*) by the method of Bendich and Bolton.[27] The DNA was purified by treatment with bovine pancrease ribonuclease A preheated to 100° for 5 min. 50 μg/ml ribonuclease was incubated with DNA for 30 min at 37°. Protein was removed by shaking with water-saturated phenol. The residual phenol was removed by washing 3 × $Et_2O$. The DNA was then precipitated with EtOH and redissolved in a small volume of 0·1 × SSC and stored at 4°.

*Thermal Transition Curves and Tm Determinations*

These were performed in 3 ml Teflon-stoppered quartz cuvettes with a 1 cm light path. The cuvettes were placed in a thermostatic chamber which permits thermostatic control of 10 cuvettes with a maximum measured temperature difference of ±0·5° among the 10 positions. To facilitate rapid handling, each cuvette was kept in a separate holder. The temperature was controlled with a Haake thermostatic bath circulating ethylene glycol, and measured with a thermister (YS1 Model 52sc Tele-Thermometer). The thermister was positioned in a blank cuvette in the thermostatic chamber, by drilling a hole through the Teflon stopper to accept the thermister lead. The ethylene glycol was circulated from the Haake thermostat into the temperature controlled cell compartment of a Cary 15 spectrophotometer which was connected to the thermostatic chamber leading the ethylene glycol back to the Haake thermostat. All the connecting tubes were insulated. The temperature drop between the thermostat and the cuvettes varied from 2° to 3° over the operation range of 25°–100°. The temperatures of a cuvette in the thermostatic chamber and a cuvette in the Cary 15 cell compartment did not vary beyond ±0·5°.

When determining the Tm, 25 μg/ml DNA and the indicated hormone concentrations, were first incubated at 25° for 30 min. The temperature of the thermostat was then raised in increments of 3°–5°. The system was maintained at a given temperature for 10 min before recording the u.v. spectrum of all samples by successively transferring them from the thermostatic chamber to the Cary 15 spectrophotometer cell compartment. After each rise of 8° the DNA-hormone mixtures were mixed by turning the cells upside down four times.

All absorbance data were corrected for thermal expansion of the solution, using the expansion coefficient of water.[28] Values of the median temperature of denaturation, Tm, were calculated by mathematical interpolation between the two points on either side of the 50 per cent absorbance increase point. All the corrections and calculations described were processed with the computer.

*Acknowledgements*—The skilful technical assistance of Mrs. Michaela Sharabani is gratefully acknowledged The author thanks D. E. Yagil and Dr. Y. Shain, Tel-Aviv University, for their critical review of this manuscript.

[27] A. J. BENDICH and E. T. BOLTON, *Plant Physiol.* **42**, 959 (1967).

[28] M. MANDEL and J. MARMUR, *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), Vol. XII (B), p. 195, Academic Press. New York (1968).