Phytochemistry, 1972 Vol. 11, pp. 3135 to 3138. Pergamon Press. Printed in England.

ROLE OF pH IN THE OBSERVED EFFECT OF PLANT GROWTH REGULATORS ON DNA*

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(*Received* 21 *March* 1972. *Accepted* 7 *June* 1972)

Key Word Index—DNA binding; plant growth regulators; mode of action of plant hormones; role of pH.

Abstract—Indole-3-acetic acid, gibberellic acid, kinetin, 6-benzylaminopurine, 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 3,6-dichloro-*o*-anisic acid, 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine and α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine do not affect the thermal denaturation or T_m of DNA by hyperchromicity measurements except as they alter the pH of the test solution due to their acidic or basic nature. Similar changes in T_m were observed by changing the pH of the test solutions to a comparable degree with acetic acid or HCl. These plant growth regulators and herbicides apparently do not influence chromatin-directed RNA synthesis by directly altering DNA to DNA bonding.

INTRODUCTION

STUDIES on the mode of action of plant hormones and growth regulators have shown these compounds to cause a multiplicity of physiological and biochemical changes in plants as exemplified by the research on 2,4-dichlorophenoxy acetic acid (2,4-D).[1] Linking the primary mode of action of these regulators to nucleic acid metabolism would seem to offer a facile explanation for many of the induced changes reported for growth regulators. Enhanced transcription as evidenced by an increase in chromatin-directed RNA synthesis has been reported for indoleacetic acid (IAA), gibberellic acid (GA), and kinetin (KIN) in cucumber,[2] for GA_3 in peas,[3] and for 2,4-D[4] and 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine (atrazine)[5] in soybean. In contrast the herbicide α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine (trifluralin) inhibited transcription in corn.[6]

The polypeptide, actinomycin D, has been shown to complex with DNA.[7] The formation of this complex inhibits the separation of the two DNA strands induced by elevated temperature or decreased pH.[8] The influence of plant growth regulators on the physical structure of DNA appears to be an attractive explanation for their observed mediation of plant processes. Bamberger[9] reported that 10^{-4} M–10^{-3} M IAA, GA, 2,4-D, and 2,4,5,-trichlorophenoxyacetic acid (2,4,5-T) lowered DNA melting temperature (T_m), implying a

* Michigan Agricultural Experimental Station Journal Article No. 5855.

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possible increase in template sites for *in vivo* transcription due to loosening of DNA to DNA binding in the double helix.

The object of this investigation was to find an explanation for the observed effect of plant growth regulation on DNA reported by Bamberger.[9]

RESULTS

The melting profiles of calf thymus, salmon sperm, and pea stem DNA in 0·02 standard saline citrate (SSC), adjusted to pH 7·0 are shown in Fig. 1. The T_m values ranged from 63·5° for calf thymus DNA to 65·5° for pea stem DNA.

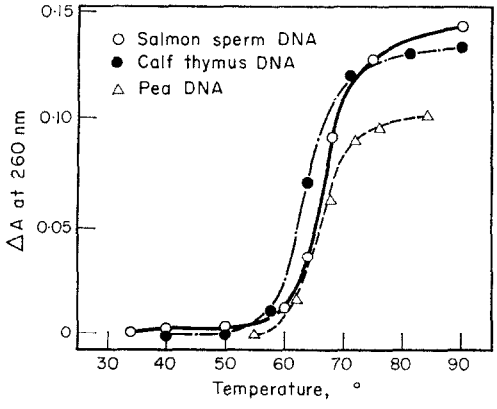

FIG. 1. THE MELTING PROFILE OF SALMON SPERM DNA, CALF THYMUS DNA, AND PEA DNA AT pH 7·0 IN 0·02 SSC.

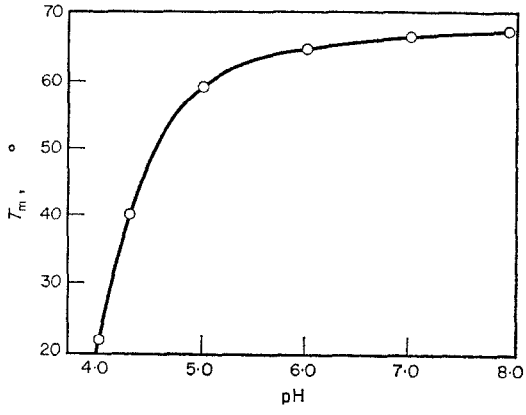

FIG. 2. THE EFFECT OF pH ON THE MELTING TEMPERATURE (T_m) OF SALMON SPERM DNA IN 0·02 SSC ADJUSTED WITH HCl.

The T_m values obtained with various plant growth regulator and herbicide solutions containing DNA in 0·02 SSC made from the pH 7·0 SSC prepared according to Bamberger[9] are shown in Table 1. The final pH of each solution was recorded and is also shown in Table 1. The depression of T_m by 10^{-3} M 2,4,-D, 10^{-4} M 2,4,5,-T, $2{\cdot}5 \times 10^{-4}$ M IAA, and 10^{-3} M GA reported by Bamberger[9] was observed. This effect on T_m was associated with a lowering of the pH of the solution due to the acidic nature of the growth regulators. Acetic acid at 10^{-2} M and 10^{-5} M 3,6-dichloro-*o*-anisic acid (dicamba) also depressed the T_m and the pH of the solution. A slight increase in T_m was observed with 10^{-4} M KIN. This was associated with an increase in the pH of the solution to 9·5.

The effect of pH on the melting temperature of salmon sperm DNA is shown in Fig. 2. The T_m values decreased with lowered pH, dropping sharply at pH values below 5.

The T_m values obtained with various growth regulator and herbicide solutions containing DNA from calf thymus, salmon sperm, or pea stem in 0·02 SSC adjusted to pH 7·0 just prior to thermal denaturation are also shown in Table 1. Under conditions where the pH of the solution was maintained at 7·0 none of the compounds examined significantly altered the T_m from the control, indicating no effect of these compounds on the thermal separation of the DNA strands.

TABLE 1. THE MELTING OF DNA IN THE PRESENCE OF VARIOUS GROWTH REGULATORS IN 0·02 SSC WITH THE pH EITHER UNADJUSTED OR AT pH 7·0

		Calf thymus DNA			Salmon sperm DNA			Pea seedling DNA
Growth regulator	Concn (M)	0·02 SSC unadjusted pH	T_m at unadjusted pH (°)	T_m at pH 7·0 (°)	0·02 SSC unadjusted pH	T_m at unadjusted pH (°)	T_m at pH 7·0 (°)	T_m at pH 7·0 (°)
Control		7·0	63·5	63·5	7·0	65·0	65·0	65·5
IAA	$2·5 \times 10^{-4}$	5·4	55·0	64·0	5·6	63·0	64·5	65·0
GA	10^{-3}	3·8	41·0	64·5	4·0	35·5	65·5	66·5
KIN	10^{-4}	9·5	67·5	62·5	9·3	66·0	63·0	64·5
6-Benzyl-aminopurine	$2·5 \times 10^{-5}$	6·8	65·0	63·5	6·6	65·0	65·5	65·0
2,4-D	10^{-3}	3·5	< 20	63·0	3·6	< 20	65·5	65·5
2,4,5-T	10^{-4}	4·7	49·5	62·5	4·6	59·0	66·0	65·5
Atrazine	10^{-5}	6·7	62·0	64·5	6·6	65·5	66·5	
Trifluralin	10^{-5}	6·5	62·5	66·5	6·6	64·0	65·0	
Dicamba	10^{-5}	3·6	< 20	62·5	3·4	< 20	68·0	
Acetic acid	10^{-4}	6·4	65·0					
	10^{-3}	5·9	63·0					
	10^{-2}	4·4	40·0					

DISCUSSION

The loosening of DNA to DNA bonds in the double helix would appear to be an attactive explanation for the reported increase in chromatin-directed RNA synthesis by various plant growth regulators and herbicides. Similarly the tightening of these bonds by these types of compounds might explain inhibition of RNA synthesis and subsequently growth.

In this study the loosening of the DNA to DNA bonds as evidence by the lowering of the T_m values in hyperchromicity measurements reported by Bamberger[9] for 10^{-3} M 2,4,-D, 10^{-4} M 2,4,5,-T, $2·5 \times 10^{-4}$ M IAA, and 10^{-3} M GA were observed when his procedures were followed. The increase in T_m by 10^{-4} M KIN was also observed. However, these procedures did not adequately maintain the pH of the solutions at 7·0. The addition of these growth regulators to the 0·02 SSC similar to that used by Bamberger[9] exceeded the buffering capacity of the buffer. All growth regulator or herbicide-induced changes in T_m values were associated with changes in pH of the solution in this study. The data in Table 1 and Fig. 1 indicate that altering the pH of the solution containing the DNA with acetic acid or HCl caused the same shift in T_m.

The data shown in Table 1 from experiments in which the DNA from a diversity of sources and plant growth regulators or solutions containing herbicide were maintained at pH 7·0, clearly indicate that none of these compounds altered DNA to DNA binding by any intrinsic structural feature generally associated with their growth regulating or herbicidal action. It is conceivable, but unlikely, that DNA might have a specific affinity for these compounds causing their accumulation in the microenvironment of the DNA to the concentration necessary to alter the pH around the DNA to the extent necessary to effect bonding changes. Rather, it appears from the data that the increase in chromatin-directed

RNA synthesis reportedly induced by IAA,[2] KIN,[2] GA,[3] 2,4-D and atrazine[5] possibly due to increased template availability, results from the primary interaction of these compounds with the nucleoprotein or other constituents of chromatin than DNA.

EXPERIMENTAL

Calf thymus DNA was obtained from Sigma. Salmon sperm DNA was obtained from Cal Bio Chem. DNA from pea seedlings was extracted from 6-day-old seedling stems after the method of Bendich and Bolton[10] and similar to the procedure reported by Bamberger.[9] The crude DNA extract was purified by dissolving it in a minimum volume of standard sodium citrate (SSC) containing 0·15 M sodium citrate adjusted to pH 7·0, adding 1/9 vol. of 5% sodium lauryl sulfate in 45% aq. EtOH, and stirring for 1 hr. Solid NaCl was then added to make a 5% solution. This solution was stirred for 0·5 hr and allowed to set overnight at 5°. The mixture was then centrifuged for 30 min at 22 000 *g* and the supernatant removed and precipitated with 2 vol. of cold absolute EtOH. The precipitated DNA was removed and dissolved in SSC and incubated with 1 mg of ribonuclease which was heated to 100° for 5 min. After reprecipitating the DNA with 2 vol. of absolute EtOH the aforementioned treatment with sodium lauryl sulfate was repeated. The final purified DNA was dissolved in 0·02 SSC.

All hyperchromiticity measurements were made in 0·02 SSC with a Perkin Elmer 139 spectrophotometer equipped with a jacketed cuvette which had ethylene glycol circulated from a controlled temperature bath through the jacket. Temperature readings were made by continuous monitoring of the temperature of the ethylene glycol leaving the cuvette. All data reported were from experiments which were repeated at least twice.

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