Short Communication

Inhibition of in vitro DNA Synthesis by Auxins[1]

Sigmund Schwimmer
Western Regional Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Albany, California 94710

Received February 12, 1968.

Recent investigations have clearly demonstrated that enhancement or repression of nucleic acid synthesis is an obligatory concomitant of the physiological response of plants to plant growth regulators. Thus the regulation of cell elongation in soybean hypocotyl by indole-3-acetic acid (IAA) accompanies and is dependent on RNA synthesis (10). 2,4-Dichlorophenoxyacetic acid (2,4-D) stimulates synthesis of ribosomal RNA (11). The gibberellins enhance messenger-like RNA synthesis in barley endosperm (22) and both DNA synthesis in and DNA content of non-dividing cells of lentil epicotyl (14). On the other hand, DNA synthesis has been reported to be suppressed by application of 2,4-D to peanut cotyledon (3, 4) and of dormin to duckweed (21). In the present investigation we find that 2,4-D and IAA, but not gibberellic acid inhibit DNA synthesis by *E. coli* DNA polymerase supported by free DNA, chromatin or nucleohistone prepared from pea embryo. The range of concentrations at which the inhibition occur suggest that interference with DNA synthesis may play a role in the toxic or herbicidal effects of these plant growth regulators.

DNA polymerase, prepared according to a modification (18) of the method Richardson *et al.* (17) through step 7, had a specific activity of 800 units per mg of protein using "activated" DNA as primer and about 180 with calf thymus DNA as primer. The chromatin fraction of both dwarf pea (var. Progress No. 9) and normal pea (var. Alaska) were prepared from 40 hour embryonic axes according to the procedure of Huang and Bonner (8). Nucleohistone was prepared from the chromatin fraction by shearing and differential centrifugation (2). Chromatin can serve as primer for both RNA and DNA polymerases, but only DNA polymerase will utilize nucleohistone as primer in a limited DNA *in vitro* synthesis (8, 18, 19). Isolation of native uncomplexed DNA from the chromatin fraction and characterization of these DNA fractions have been previously described (2, 18). Each ml of the DNA polymerase reaction mixture contained: potassium phosphate buffer pH 7.2, 40 μmoles; $MgCl_2$, 4 μmoles; 2-mercaptoethanol, 0.6 μmole; deoxynucleoside triphosphates of guanosine, thymidine, cytidine and adenosine (^{3}H-labeled, Schwarz, 10 μc/μmole), 0.02 μmole each; DNA polymerase, 1.3 units (calf thymus DNA as primer). The primer concentrations in nucleotide equivalents of DNA (18) were 0.02, 0.29, 0.10, and 0.20 μmole per ml for DNA, nucleohistone, pea chromatin and dwarf pea chromatin, respectively. DNA synthesized was measured by the incorporation of ^{3}H-dAMP into perchloric acid-insoluble material (18). "Enzyme after" controls were run. The rates are presented as mμmoles of DNA synthesized [dAMP incorporated $\times$ 3.6 (18)] per ml reaction mixture per 30 minutes at 37°. The plant growth regulators were obtained from Cal Biochem.

Figure 1 shows the effect of adding 2,4-D, IAA and GA to DNA polymerase reaction mixtures. Both 2,4-D and IAA inhibit *E. coli* DNA polymerase. The inhibition is manifested in the presence of DNA, nucleohistone, or chromatin primers. When calculated on the basis of percentage inhibition, inhibition by both 2,4-D and IAA was most efficient in the presence of chromatin and least efficient in the presence of free DNA. Thus in the presence of 0.15 mM 2,4-D, the interpolated percentage inhibition with DNA, nucleohistone and chromatin as primers are 40, 51, and 81, respectively. Similarly, in the presence of 0.5 mM IAA the corresponding percentage inhibitions were 33, 48, and 58, respectively.

It is not possible from the data presented to ascertain the nature of the variation of the different inhibitory effects in the presence of these primers. On the basis of simple Michaelis kinetics, differences in K_m and K_i values, but not in initial primer concentration, could account for the variations. It has been suggested that some plant growth regula-

[1] This work was conducted under a cooperative agreement between the Division of Biology, California Institute of Technology, and the Western Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture. Report of work supported in part by United States Public Health Service Grants GM-03977, GM-05143, AM-03102. Reference to a company or product name does not imply approval or recommendation of the product by the United States Department of Agriculture to the exclusion of others that may be suitable.

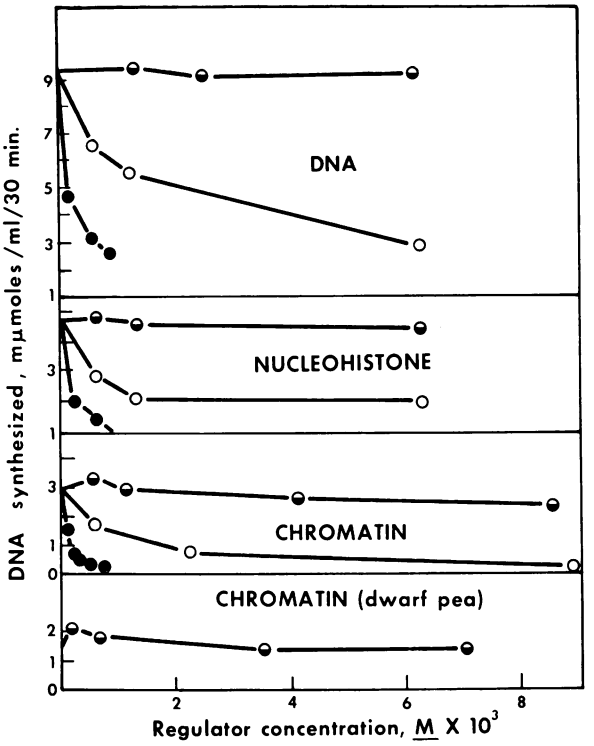

FIG. 1. Effect of the concentration 2,4-dichlorophenoxyacetic acid (●), indole-3-acetic acid (○) and gibberellic acid (half-closed circle) on *E. coli* DNA polymerase with DNA, nucleohistone and chromatin of normal peas and the chromatins of dwarf pea as primers.

tors may act as allosteric effectors of DNA polymerase (21). On the other hand, the IAA inhibition may be due, as in the case of the indole-derived antibiotic mitomycin (23), to formation of a polymerase-resistant complex with DNA. The inhibition by 2,4-D may be related to the specific inhibition of *in vivo* DNA synthesis by phenethyl alcohol (1).

Are the data on the inhibition by 2,4-D and IAA pertinent to the physiological action of plant growth regulators? The inhibition by IAA occurs at concentrations several orders of magnitude greater than concentrations at which growth regulator effects and nucleic acid synthesis enhancement are manifested. Thus the interpolated millimolar concentrations of IAA for 50 % inhibition with DNA nucleohistone and chromatin are 2.1, 1.1 and 0.7, respectively.

Inhibition of DNA polymerase by 2,4-D appeared to be substantial in a concentration range of about 1 order of magnitude (10^{-4}–10^{-5} M) lower than the range in which IAA was effective. This is still much higher than the concentrations (10^{-7}–10^{-8} M) at which 2,4-D, when applied to plants, stimulates growth. It is however in the same range of concentration found for the inhibition of DNA synthesis. Thus, at 1.25×10^{-5} M 2,4-D caused a 12 % inhibition of DNA synthesis in peanut cotyledon as measured by the incorporation of ^{32}P (3).

Although the molecular basis of herbicidal action has been the subject of a great deal of speculation and controversy (5, 7, 12), only recently have suggestions been forwarded that DNA polymerase inhibition may be involved (3, 13, 18). It should be pointed out that it may be necessary to apply these herbicides in a concentration range a few orders of magnitude greater than those used in these experiments in order to attain comparable levels at their putative locus of action, presumably nuclear DNA. We suggest that the inhibition of DNA polymerase by very high concentrations of auxins may in part be responsible and lead to their herbicidal effects. Thus inhibition of chloroplast DNA polymerase (20) could lead to accumulation of phosphate esters (15), higher metabolic rates and chlorosis (16).

Gibberellic acid did not, within experimental error (± 5 %) influence the activity of DNA- or nucleohistone-supported DNA polymerase. With normal pea chromatin as primer there appeared to be a barely perceptible increase at concentrations between 10^{-3} M and a slight inhibition at about 10^{-2} M. In the presence of dwarf pea chromatin a 25 % increase in polymerase activity was observed at 10^{-4} M gibberellic acid. It is of interest to note that gibberellin applied at concentrations of about 3×10^{-4} M promotes a 35 % enhancement of DNA synthesis in elongating, non-dividing cells of lentil epicotyl (14). Some, but not all, sex steroids have been reported to cause small (*ca.* 10 %) stimulation of mammalian DNA polymerase (6). On the other hand, synthesis of DNA-like RNA in isolated nuclei of dwarf peas was enhanced as much as 100 % in the presence of 10^{-8} M gibberellic acid (7).

Literature Cited

1. BERRAH, G. AND W. A. KONETZKA. 1962. Selective and reversible inhibition of the synthesis of bacterial deoxyribonucleic acid by phenethyl alcohol. J. Bacteriol. 83: 738–44.
2. BONNER, J. AND R. C. C. HUANG. 1963. Properties of chromosomal nucleohistone. J. Mol. Biol. 6: 169–74.
3. CARPENTER, W. J. G. AND J. H. CHERRY. 1966. Effects of protein inhibitors and auxins on nucleic acid metabolism in peanut cotyledons. Plant Physiol. 41: 919–22
4. CHERRY, J. H. 1967. Nucleic acid biosynthesis in seed germination: Influences of auxin and growth regulating substance. Ann. N. Y. Acad. Sci. 144: 154–68.
5. CRAFTS, A. S. 1961. The chemistry and mode of action of herbicides. Interscience Publishers Inc., New York.
6. FAHMY, A. R., K. GRIFFITHS, R. MAHLER, AND A. R. WILLIAMS. 1967. The effect of steroids on deoxyribonucleic acid polymerase. Biochem. J. 105: 6C–7C.
7. HILTON, J. L., L. L. JANSEN, AND H. M. HULL. 1963. Mechanisms of herbicide action. Ann. Rev. Plant Physiol. 14: 353–84.

8. HUANG, R. C. C. AND J. BONNER. 1962. Histone, a suppressor of chromosomal RNA synthesis. Proc. Natl. Acad. Sci. 48: 1216–22.
9. JOHRI, M. M. AND T. E. VARNER. 1967. Enhancement by gibberellins of RNA synthesis in isolated nuclei. Plant Physiol. 42: xi.
10. KEY, J. L., N. M. BARNETT, AND C. Y. LIN. 1967. RNA and protein synthesis and the regulation of cell elongation by auxins. Ann. N. Y. Acad. Sci. 144: 49–62.
11. KEY, J. L. AND J. C. SHANNON. 1964. Enhancement by auxin of ribonucleic acid synthesis in excised soybean hypocotyl tissue. Plant Physiol. 39: 360–64.
12. MANN, J. O., L. S. JORDAN, AND B. E. DAY. 1963. A survey of herbicides for their effect on protein synthesis. Plant Physiol. 40: 840–43.
13. MORELAND, D. E. 1967. Mechanisms of action of herbicides. Ann. Rev. Plant Physiol. 18: 365–86.
14. NITSAN, J. AND J. LANG. 1966. DNA synthesis in the elongating non-dividing cells of lentil epicotyl and its promotion by gibberellin. Plant Physiol. 41: 965–70.
15. OMROD, D. P. AND W. A. WILLIAMS. 1960. Phosphorus metabolism of *Trifolium hirtum all.* as affected by 2,4-dichlorophenoxyacetic acid and gibberellin. Plant Physiol. 35: 81–87.
16. OSBORN, D. J. AND M. HALLAWAY. 1961. Plant growth regulation. Iowa State University Press, Ames, Iowa. p 329–39.
17. RICHARDSON, C. C., C. L. SCHILDKRAUT, H. U. APOSHIAN, AND A. KORNBERG. Enzymatic synthesis of deoxyribonucleic acid. XIV. Further purification and properties of deoxyribonucleic acid polymerase of *Escherichia coli.* J. Biol. Chem. 229: 222–32.
18. SCHWIMMER, S. 1967. Susceptibility to nucleases of DNA synthesized with nucleohistone primer. Biochim. Biophys. Acta 134: 59–68.
19. SCHWIMMER, S. AND J. BONNER. 1965. Nucleohistone as template for the replication of DNA. Biochim. Biophys. Acta 108: 67–72.
20. TEWARI, K. K. AND S. G. WILDMAN. 1967. DNA polymerase in isolated tobacco chloroplasts and the nature of the polymerized product. Proc. Natl. Acad. Sci. 50: 689–96.
21. VAN OVERBEEK, J., J. E. LOEFFLER, AND M. I. R. MASON. 1967. Dormin (Abscisin II), inhibitor of plant DNA synthesis. Science 156: 1497–99.
22. VARNER, J. E. 1967. Hormonal control of enzyme production in barley endosperms. Ann. N. Y. Acad. Sci. 144: 219–22.
23. ZYBALSKI, W. AND V. N. IYER. 1964. Crosslinking of DNA by enzymatically or chemically activated mitomycins and porfiromycins, bifunctional "alkylating" antibiotics. Federation Proc. 23: 946–57.