Plant Physiol. (1966) 41, 919–922

Effects of Protein Inhibitors and Auxin on Nucleic Acid Metabolism in Peanut Cotyledons

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Received January 5, 1966.

Summary. The accumulation of labeled phosphorus into newly synthesized nucleic acids or peanut cotyledon slices incubated with chloramphenicol, puromycin, or 2,4-dichlorophenoxyacetic acid (2,4-D) was reduced. Promotion of nucleic acid synthesis was not noted by any of these chemicals. Chloramphenicol completely inhibited the synthesis of the DNA-RNA fraction at 1.25×10^{-3} M while soluble and ribosomal RNA was inhibited by 70 % and 80 %, respectively. At the same concentration messenger RNA was inhibited by only 40 %. These effects suggest that chloramphenicol inhibit nucleic acid synthesis in peanut cotyledons in a differential manner. Similar results were noted for DNA at low concentrations of 2,4-D. However, at high concentrations of 2,4-D, DNA as well as RNA fractions were inhibited in a similar manner at a given concentration. Puromycin did not differentially inhibit nucleic acid synthesis except at 2×10^{-3} M where DNA was least inhibited.

Nondifferential inhibition suggests that a common site or precursor pool essential for the synthesis of all nucleic acid fractions is altered. Differential inhibition may be due to the interference with a specific rate-limiting step, directly or indirectly, in the formation of a particular nucleic acid.

RNA synthesis in peanut cotyledons is initiated at the onset of germination and the content doubles in the first week after planting (7). Subsequently, the RNA content rapidly declines with aging of the cotyledon. Concomitent with this change in RNA content, the activities of many glycolytic and mitochondrial enzymes follow similar patterns (7). Because nucleic acids and enzymes are synthesized as judged by increased activity during germination without cell division, we selected the peanut cotyledon to study the effects of various protein inhibitors (chloramphenicol and puromycin) and auxin (2,4-dichlorophenoxyacetic acid) on nucleic acid metabolism during the early stages of germination. Any differences in action of these compounds on RNA metabolism could then be used to study in greater detail the role of this nucleic acid in plant growth and development.

Chloramphenicol and puromycin have been shown to inhibit protein synthesis in bacterial and mammalian systems by affecting the formation of the polypeptide chain (1, 4, 15,19). Also, chloramphenicol and puromycin have been shown to affect RNA synthesis (2, 10, 13, 16). Gale and Folks (10) and Kurland and Maale (13) reported that high concentrations of chloramphenicol inhibited RNA synthesis. However, Aronson and Spiegelman (2) did not observe an inhibition of RNA synthesis in *E. coli* with chloramphenicol. Puromycin inhibits the synthesis of 30S and 18S ribosomal RNA and ribosomal precursor RNA in mammalian cells (11) while soluble and ribosomal RNA accumulate in bacteria treated with puromycin (16). In our studies on nucleic acid metabolism in plants, it was felt desirable to determine whether chloramphenicol and puromycin inhibit RNA synthesis in a manner similar to that previously reported in bacterial or mammalian cells.

2,4-D also affects the synthesis of nucleic acids in higher plants primarily by promoting ribosomal RNA synthesis (12). Auxin may promote RNA synthesis at low concentrations or inhibit at high concentrations (3). Normally, auxin manifests its effect in growing tissue; however, since peanut cotyledons synthesize nucleic acids, it seemed desirable to determine if auxin influences nucleic acid synthesis in storage parenchyma cells of the peanut cotyledon.

Materials and Methods

Plant Material. Virginia 56-R peanut (*Arachis hypogaea* L.) seeds were germinated on moist absorbant paper at a high relative humidity in a dark chamber at 28° for 3 days. Ten g of sliced (1–2 mm) peanut cotyledons were preincubated for 30 minutes

[1] Journal paper 2706 of the Purdue Agriculture Experiment Station. This investigation was supported in part by a David Ross Research fellowship from the Purdue Research Foundation and a National Science Foundation Grant GB-622.

in a solution of 10^{-4} M citric acid, pH 6.0 with NH_4OH, 1 % sucrose and either chloramphenicol, puromycin, or 2,4-D. Radioactive phosphorus (0.8 mc) was then added to the incubation solution and the cotyledons were incubated for an additional 2 hours. After incubation the tissue was washed with cold water and kept on ice until used.

Nucleic Acid Extraction and Chromatography on MAK Columns. Nucleic acids were extracted from the labeled tissue in the presence of cold phenol and 2 % dupanol as previously described (8). Purified samples of nucleic acids labeled with ^{32}P were absorbed on methylated albumin coated on kieselguhr (MAK) columns according to the method of Mandell and Hershey (14) and eluted with a linear gradient of NaCl from 0.35 M to 1.15 M in 0.05 M phosphate buffer (pH 6.7). Consecutive 5 ml fractions of the eluate were collected; the ultraviolet absorbancy (260 mμ) and radioactivity were determined for each fraction.

Inhibition of nucleic acid synthesis in peanut cotyledons treated with various concentrations of chloramphenicol, puromycin, and 2,4-D is expressed as percent of control.

Results

It has been shown that cells of peanut cotyledons synthesize RNA during seed germination (6) and can be separated into 6 fractions by MAK chromatography (fig 1 A). The soluble RNAs (peaks I and II) of peanut cotyledons are separated into 2 fractions (s_1 and s_2) as characterized by Chroboczek and Cherry (9). The DNA fraction (peak III) consists of rapidly metabolized DNA, RNA and nonmetabolic DNA as characterized by Cherry (7) and will be called the DNA-RNA fraction in this paper. Ribosomal RNA is fractionated into 2 peaks (IV and V) and are termed light (lr) and heavy (hr) ribosomal RNA, respectively. These 2 RNAs are equivalent to the 16S and 23S RNA of bacteria (20) and in peanut cotyledons are found exclusively in the ribosomes (9). The last fraction (peak VI) eluted from the MAK column is termed messenger RNA (mRNA). Present data from this laboratory show that this fraction (mRNA) hybridizes with homologous DNA to a much greater extent than any other RNA fraction of peanut cotyledons. (Unpublished data of R. B. van Huystee and J. H. Cherry).

To evaluate the effects of chloramphenicol, puromycin, and 2,4-D on nucleic acid metabolism, peanut cotyledon slices were incubated with various concentrations of the chemical. The nucleic acids were extracted and fractionated by MAK chromatography. Typical ultraviolet and radioactivity profiles of the nucleic acids extracted from control, chloramphenicol, and puromycin treated tissues are presented in figure 1A, 1B, and 1C, respectively. The relative amount of total ^{32}P incorporated into the nucleic acids of untreated tissues (fig 1 A) was 10 % for each s_1RNA and DNA-RNA, 3 % for s_2RNA, 16 % for lrRNA, 42 % for hrRNA, and 19 % for mRNA. Chloramphenicol (1.25×10^{-3} M) reduced total ^{32}P incorporation into the nucleic acids by 84 %. Major reduction occurred in soluble RNA and DNA as shown by the radioactivity profiles (fig 1 B). Ribosomal RNA was inhibited more by chloramphenicol than was mRNA. Puromycin at 2.5×10^{-4} M caused a 22 % reduction of total ^{32}P incorporation into the nucleic acids. However, incorporation was not specifically reduced in any particular nucleic acid fraction (fig 1 C).

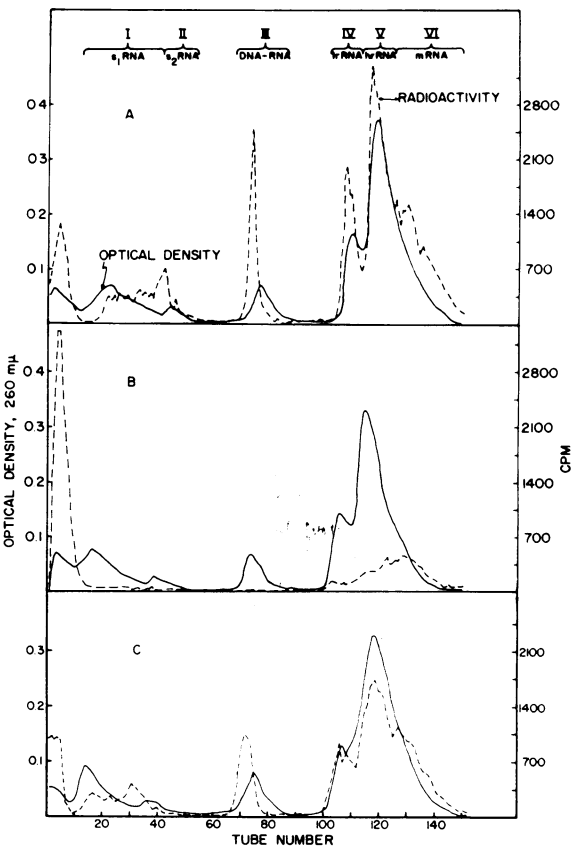

FIG. 1. Elution profiles of nucleic acids from chloramphenicol and puromycin treated peanut cotyledon slices. Sliced cotyledons from 3 day-old peanut (*Arachis hypogaea* L.) seedlings were preincubated for 30 minutes in a preincubation medium which contained various concentrations of chloramphenicol, puromycin, or 2,4-D (not shown). Radioactive phosphorus was added to the medium and samples were incubated for an additional 2 hours. Nucleic acids were extracted by a cold phenol-2 % dupanol method (8). Purified nucleic acids were separated by MAK fraction. Ultraviolet absorbancy (260 mμ) (——) and radioactivity (- - - -) from consecutive 5 ml fractions of nucleic acids from control, chloramphenicol (1.25×10^{-3} M), and puromycin (2.5×10^{-4} M) treated tissues are presented in A, B, and C, respectively. Six fractions of nucleic acids are obtained by MAK fraction: 2 soluble RNAs (s_1 and s_2), DNA-RNA, light (lr) and heavy (hr) ribosomal RNAs, and messenger RNA (mRNA).

Less nucleic acid was synthesized by chloramphenicol, puromycin, and 2,4-D-treated tissues at all concentrations tested than by nontreated tissues (fig 2). Even though less nucleic acid was synthesized in chloramphenicol treated tissues, all fractions of nucleic acid were not equally inhibited (fig 2 A). The DNA-RNA fraction was inhibited by 97 % except at 6.25×10^{-4} M. However, the inhibition of messenger RNA synthesis increased progressively from 36 % to 81 % with increased concentrations of chloramphenicol. Soluble and ribosomal RNA synthesis, on the other hand, was inhibited in the same manner.

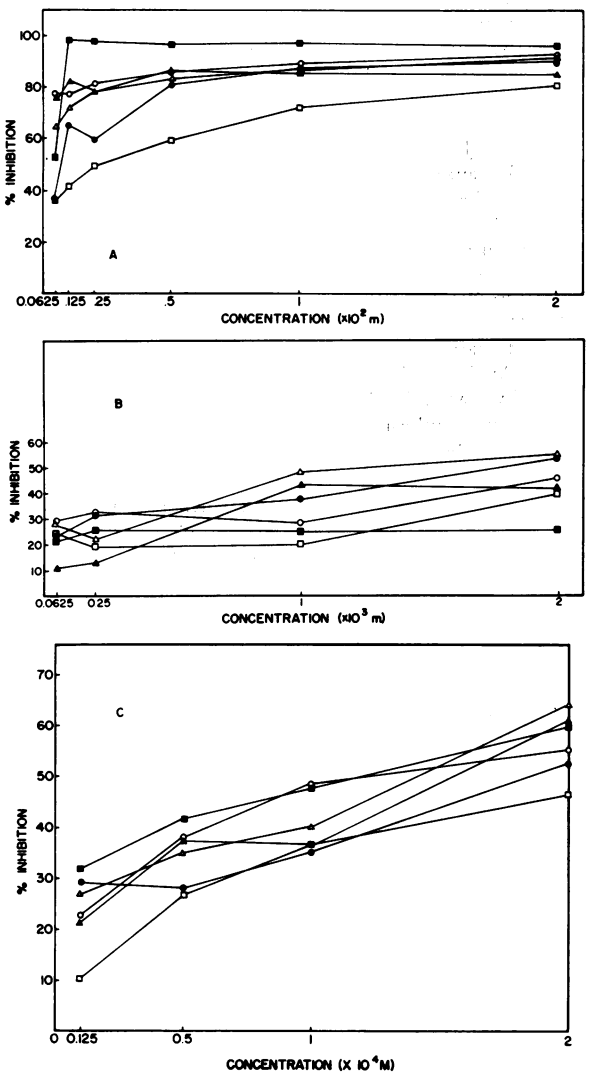

FIG. 2. Inhibition of nucleic acid synthesis of peanut cotyledon slices treated with chloramphenicol, puromycin, and 2,4-D. Peanut cotyledons were treated as described in figure 1. Inhibition of nucleic acid synthesis at various concentrations of chloramphenicol (A), puromycin (B), and 2,4-D (C) is expressed as percent of control. The nucleic acid fractions are represented by the following symbols: ●, soluble$_1$RNA; ▲, soluble$_2$RNA; ■, DNA-RNA; ○, light ribosomal RNA; △, heavy ribosomal RNA; and □, messenger RNA.

Synthesis of the DNA-RNA fraction was inhibited much less in puromycin treated tissue than at the same concentration of chloramphenicol (fig 2 B). At a given concentration of puromycin, synthesis of soluble, ribosomal, and messenger RNA was inhibited to approximately the same extent.

Messenger RNA synthesis in peanut cotyledon slices was not inhibited as much as soluble and ribosomal RNA at low concentration (1.25×10^{-5} M) of 2,4-D (fig 2 C). However, at higher concentrations, all nucleic acid fractions including DNA were inhibited about the same. While the relative synthesis of DNA appears to be inhibited more at low concentration (1.25×10^{-5} M) of 2,4-D than the RNA fractions, it is a much smaller inhibition than that caused by chloramphenicol (fig 2 A).

Discussion

This study shows that chloramphenicol and puromycin at the concentrations tested inhibit nucleic acid synthesis in peanut cotyledon slices. However, their effects on synthesis of various nucleic acid fractions are different. Synthesis of the DNA-RNA fraction in tissue treated with chloramphenicol was inhibited to a much greater extent than the RNA fractions; surprisingly the synthesis of messenger RNA was inhibited very little. Puromycin, on the other hand, progressively inhibited the synthesis of all nucleic acid fractions with increasing concentration. These data differ from those of Holland (11) and Tamaoki and Mueller (18) who reported that puromycin inhibited the synthesis of transfer and messenger RNA less than ribosomal and ribosomal precursor RNA in HeLa cells.

Spector and Kinochita (17) suggested that puromycin may affect protein synthesis not only by interfering with the template but also through the inhibition of RNA synthesis. Our data indicate that puromycin may affect nucleic acid synthesis at a common site, since all RNA fractions were inhibited to about the same extent. Perhaps, puromycin inhibits the synthesis of a common nucleotide precursor or at some other step that is essential for the synthesis of nucleic acids. On the other hand, our data show that chloramphenicol does not affect synthesis of all nucleic acids at a common site since differential inhibition occurred. But chloramphenicol inhibits the synthesis of soluble and ribosomal RNA at each concentration to about the same extent which suggests that their inhibition may occur at a common site. Present data from our laboratory (5) show that synthesis of nucleic acids in 5-fluorouracil-treated tissue is also differentially inhibited. 5-Fluorouracil, like chloramphenicol, greatly inhibits the synthesis of DNA while messenger RNA is least inhibited. Soluble and ribosomal RNA were inhibited similarly at each concentration tested. (Further details concerning these studies will be published).

Experimental evidence has shown that 2,4-D affects nucleic acid metabolism of hypocotyl sections primarily by promoting the synthesis of ribosomal RNA (12). However, peanut cotyledon slices, a storage tissue, synthesized less nucleic acid in the presence of 2,4-D. Low concentration (1.25×10^{-5} M) of 2,4-D inhibited nucleic acid synthesis by approximately 12 %. However, the main effect at low concentrations was on the DNA-RNA fraction. Higher concentrations of 2,4-D inhibited incorporation of ^{32}P into all nucleic acid fractions to about the same extent. This suggests that low concentrations of 2,4-D, like chloramphenicol, may differentially affect the synthesis of certain nucleic acids while at high concentrations this type of inhibition is not observed.

At the present time, the mechanisms of inhibition are not completely understood. Possible sites of action might include: A) inhibition of purine and pyrimidine biosynthesis, B) inhibition of phosphorylation of ribose and nucleosides pyrophosphate, or C) inhibition of enzymes involved in nucleic acid biosynthesis.

Acknowledgment

The authors thank the Parke, Davis and Company, Ann Arbor, Michigan for the gift of chloramphenicol.

Literature Cited

1. Allen, D. W. and P. G. Zamecnik. 1962. The effect of puromycin on rabbit reticulocyte ribosomes. Biochem. Biophys. Acta 55: 865–74.
2. Aronson, A. I. and S. Spiegelman. 1961. Protein and ribonucleic acid synthesis in a chloramphenicol-inhibited system. Biochem. Biophys. Acta 53: 70–84.
3. Basler, E. and T. L. Hansen. 1964. Some effects of 2,4-dichlorophenoxyacetic acid and sucrose on orotic acid uptake in nucleic acids of excised cotton cotyledons. Botan. Gaz. 125: 50–55.
4. Cannon, M., R. Krug, and W. Gilbert. 1963. The binding of *s*-RNA by *Escherichia coli* ribosomes. J. Mol. Biol. 7: 360–78.
5. Carpenter, W. J. G. and J. H. Cherry. 1965. Mechanism of action of 5-fluorouracil on nucleic acid synthesis in peanut cotyledons. Plant Physiol. 40: lxiii.
6. Cherry, J. H. 1963. Nucleic acid, mitochondria, and enzyme changes in cotyledons of peanut seeds during germination. Plant Physiol. 38: 440–46.
7. Cherry, J. H. 1964. Association of rapidly metabolized DNA and RNA. Sci. 146: 1066–69.
8. Cherry, J. H., H. Chroboczek, W. J. G. Carpenter, and A. Richmond. 1965. Nucleic acid metabolism in peanut cotyledons. Plant Physiol. 40: 582–87.
9. Chroboczek, H. and J. H. Cherry. 1966. Characterization of nucleic acids in peanut cotyledons. J. Mol. Biol. In Press.
10. Gale, E. F. and J. P. Folks. 1953. The assimilation of amino acids by bacteria. 15. Actions of antibiotics on nucleic acid and protein synthesis in *Staphylococcus aureus*. Biochem. J. 53: 493–98.
11. Holland, J. J. 1963. Effects of puromycin on RNA synthesis in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. 50: 436–43.
12. 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.
13. Kurland, C. G. and O. Maale. 1962. Regulation of ribosomal and transfer RNA synthesis. J. Mol. Biol. 4: 193–210.
14. Mandell, J. D. and A. D. Hershey. 1960. A fractionation column for analysis of nucleic acids. Anal. Biochem. 1: 66–77.
15. Nathans, D. 1964. Puromycin inhibition of protein synthesis: Incorporation of puromycin into peptide chains. Proc. Natl. Acad. Sci. U.S.A. 51: 585–92.
16. Sells, B. H. 1965. Puromycin: Effect on messenger RNA synthesis and β-galactosidase formation in *Escherichia coli* 15T. Sci. 148: 371–73.
17. Spector, A. and J. H. Kinochita. 1965. The effect of actinomycin D and puromycin upon RNA and protein metabolism in calf lens. Biochem. Biophys. Acta 95: 561–68.
18. Tamaoki, T. and G. C. Mueller. 1963. Effect of puromycin on RNA synthesis in HeLa cells. Biochem. Biophys. Res. Commun. 11: 404–10.
19. Wolfe, A. D. and F. E. Hahn. 1965. Mode of action of chloramphenicol upon a ribosomal amino acid polymerization system and its binding to bacterial ribosome. Biochem. Biophys. Acta 95: 146–55.
20. Yankofsky, S. A. and S. Spiegelman. 1963. Distinct cistrons for the 2 ribosomal RNA components. Proc. Natl. Acad. Sci. U.S.A. 49: 538–44.