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THE APPEARANCE OF PYRIMIDINE NUCLEOSIDE AND DEOXYNUCLEOSIDE KINASE ACTIVITIES IN PEANUT AXES*

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Abstract—The appearance of pyrimidine nucleoside and deoxynucleoside kinase activities in peanut axes was followed during the first 96 hr of germination. The activation or synthesis of these enzymes preceded increases in RNA, DNA and fresh weight. The incorporation of DNA precursors into DNA occurred only after the formation of the pyrimidine deoxynucleoside kinases.

INTRODUCTION

THE APPEARANCE of pyrimidine nucleoside and deoxynucleoside kinase activity has been demonstrated in only a few plants.[1,2] Wanka and Bauer[3] suggested that one aspecific kinase rather than several kinases was responsible for pyrimidine nucleoside phosphorylation during the early growth of corn seedlings. They indicated that the appearance of the aspecific kinase activity could be correlated with an increase in soluble nucleotides in the seedling. This correlation suggests a major synthetic role for the aspecific nucleoside kinase in providing the immediate precursors needed for the synthesis of nucleic acids during this stage of development.

Hotta and Stern[1,4] demonstrated a precise switching on and off of thymidine kinase activity in developing lily microspores just prior to an increase in the DNA content of the cell. In bacteria and various mammalian cell culture systems, thymidine kinase activity has been shown to be closely associated with the biochemical events culminating in DNA synthesis[5] and the rapid proliferation of cells.[6–11] Increases in thymidine kinase activity have also been noted following viral infection of mammalian tissues.[12] Consequently, such evidence suggests that the appearance and/or increase of thymidine kinase activity is closely linked to DNA synthesis and subsequent cell division.

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In plants, however, the evidence which links the appearance of pyrimidine nucleoside and deoxynucleoside kinase activities to an increase in the nucleic acid content of developing seedlings is still limited. Thus, the implication for the necessity of these kinases and particularly of thymidine kinase for DNA synthesis led us to examine the biochemical and physiological events involved in the formation of these constituents during the early germination of peanut seedlings. These findings are described in this report.

RESULTS AND DISCUSSION

Since previous work has shown that varying levels of thymidine kinase activity can be correlated closely with changes in rates of DNA synthesis and cell division,[1,6,8,13] we attempted to ascertain if any biosynthetic patterns involving thymidine kinase and associated pyrimidine nucleoside and deoxynucleoside kinases could be identified during the initial 96 hr of imbibition and germination of peanut seedlings. Peanut axes (25/replication/sampling period) were harvested at intervals of 12 hr during this period. Phosphorylation of various pyrimidine nucleosides and deoxynucleosides by enzyme extracts prepared after various periods of germination is shown in Figs. 1 and 2. We have previously reported[14] that partially

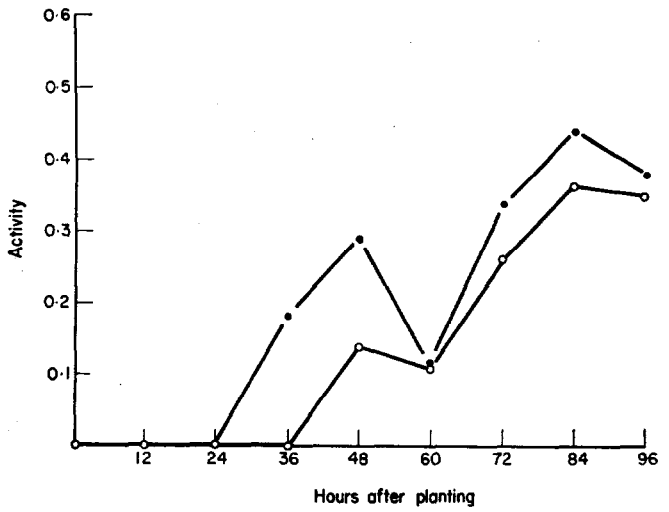

FIG. 1. URIDINE AND CYTIDINE KINASE ACTIVITIES DURING GERMINATION.
Peanut axes (25/sample period) were treated with NaOCl (1·5% for 10 min) before grinding in phosphate buffer (0·2 M, pH 7·0) in a pre-chilled mortar and pestle (6 ml buffer/25 seedlings). The preparation was then passed through several layers of fine nylon cloth and centrifuged at 32,000 g for 30 min. The supernatants were then assayed in the usual manner. Activities (% phosphorylation/min) are an average of eight determinations. (●—●, Uridine kinase; ○—○, cytidine kinase.)

purified preparations from peanut seedlings had the ability to phosphorylate the various pyrimidine nucleosides and deoxynucleosides indicated in Figs. 1 and 2. Because of the presence of strong nucleoside phosphatase activity in all of our preparations the activity values shown are net and not absolute.[14] However, this problem does not necessarily vitiate the interpretation of our observations.

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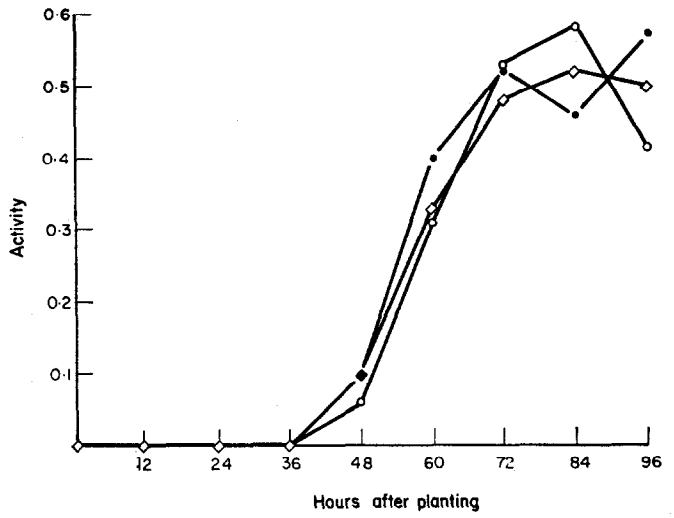

FIG. 2. THYMIDINE, DEOXYURIDINE AND DEOXYCYTIDINE KINASE ACTIVITIES DURING GERMINATION. Protocol as in Fig. 1 (legend). Activities (% phosphorylation/min) are an average of eight determinations. (○—○, Deoxyuridine kinase; ●—●, thymidine kinase; ◇—◇, deoxycytidine kinase.)

With the exception of uridine, phosphorylation of the various pyrimidine nucleosides and deoxynucleosides was not detectable until 36–48 hr after initiation of germination (Figs. 1 and 2). The reason for decreased uridine phosphorylation at 60 hr is unknown although as indicated in Fig. 1, cytidine phosphorylation followed a somewhat similar pattern. If real, such a result might be attributable to transient fluctuations in ribonucleotide phosphatase activities or in the case of uridine, the appearance of two different uridine kinases[15] might be responsible.

Comparison of pyrimidine nucleoside and deoxynucleoside kinase activities (Figs. 1 and 2) with fresh weight increments and nucleic acid content (Fig. 3) indicates that cell expansion and nucleic acid synthesis increased rapidly after the appearance of these enzymes. RNA content began to increase approximately 12 hr after the appearance of uridine kinase activity and paralleled the appearance of the other nucleoside kinases. Inspection of Fig. 2 indicates the similarity of all the pyrimidine deoxynucleoside kinase profiles. Whether in fact we have recorded the induction or activation of several specific pyrimidine deoxynucleoside kinases or a single kinase capable of phosphorylating any deoxypyrimidine nucleoside is not known.[14] Wanka and Bauer[3] present evidence that the latter is true for corn seedlings.

The enzyme or enzymes capable of phosphorylating pyrimidine deoxynucleosides were produced (Fig. 2) approximately 24 hr before changes in DNA content (Fig. 3) could be detected. However, colorimetric estimates of DNA content do not accurately reflect synthetic patterns or offer the necessary resolution for determining the initiation of synthesis. The following experiments were designed to determine if the onset of incorporation of nucleic acid precursors coincided with the appearance of the above enzymes.

In order to obtain an estimate of thymidine utilization, ^{14}C-thymidine incorporation into perchloric acid (PCA) soluble and insoluble fractions[16] was followed before (24 hr) during (42 hr) and after (60 hr) appearance of thymidine kinase activity in axes of germinating

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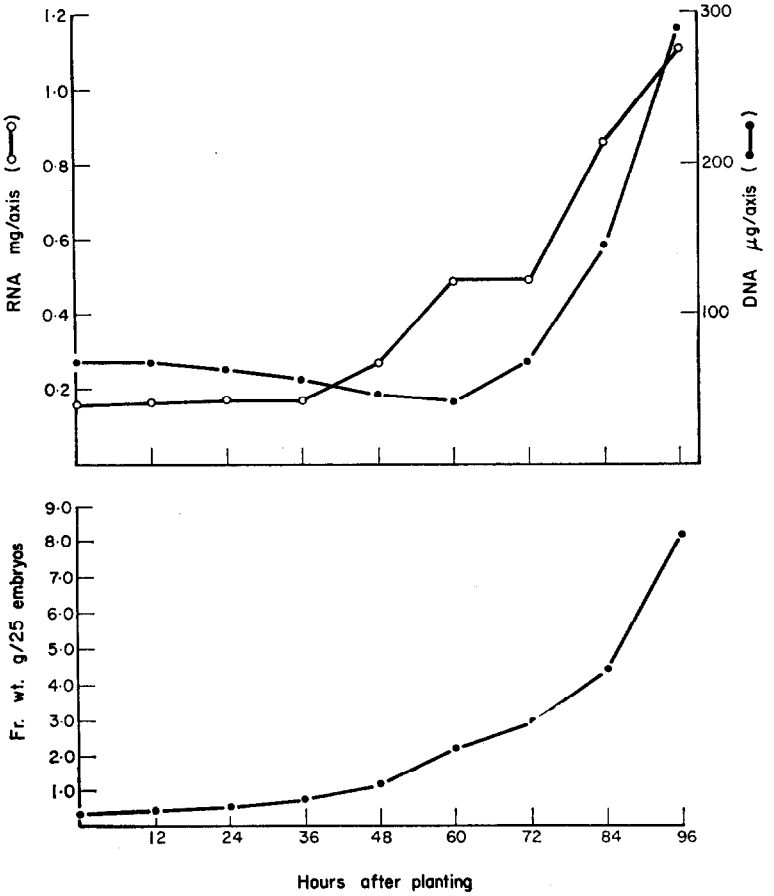

FIG. 3. NUCLEIC ACID AND FRESH WEIGHT CHANGES DURING GERMINATION.
Groups of 25 axes were harvested at the times indicated, weighed for the fresh weight determination and immediately frozen until all samples were collected. RNA and DNA determinations were made as in Experimental. Each point is an average of at least four determinations.

peanuts (Table 1). Total counts (PCA soluble plus PCA insoluble fractions) decreased with increasing seedling age. Relative and specific activities (Table 1) were used as estimates of thymidine incorporation into DNA. Although the PCA insoluble counts can not be construed

TABLE 1. ISOTOPIC THYMIDINE UTILIZATION BY GERMINATING PEANUT AXES

Hours germinated	Total counts ($\times 10^{-5}$)	Relative activity	Specific activity
24	2·6	0·04	6·24
42	2·2	0·12	23·9
60	1·7	0·19	28·3

Axes were harvested at the respective times and incubated for 4 hr in 10 μc ^{14}C-thymidine in water. Total counts equal PCA soluble plus PCA insoluble fractions. Relative activity equals PCA insoluble CPM divided by total counts. Specific activity equals PCA insoluble CPM/μg DNA.

solely as incorporation into DNA, there is apparently very little diversion of thymidine into RNA in plants.[17] Thus as shown in Table 1, the efficiency of thymidine incorporation into the insoluble fraction as well as the specific activities increased approximately three and five-fold (42 and 60 hr, respectively) when thymidine kinase activity was present compared to axes devoid (24 hr) of the kinase.

The incorporation of carrier-free ^{32}P was similarly followed after 24, 54, and 72 hr germination periods. Axes were excised at the respective periods of germination and incubated in isotope before extraction and separation of the nucleic acid fractions (see Experimental). Axes germinated for 24 hr failed to incorporate ^{32}P into DNA; however these axes synthesized some RNA as indicated by the label present in the ribosomal RNA regions (Fig. 4).

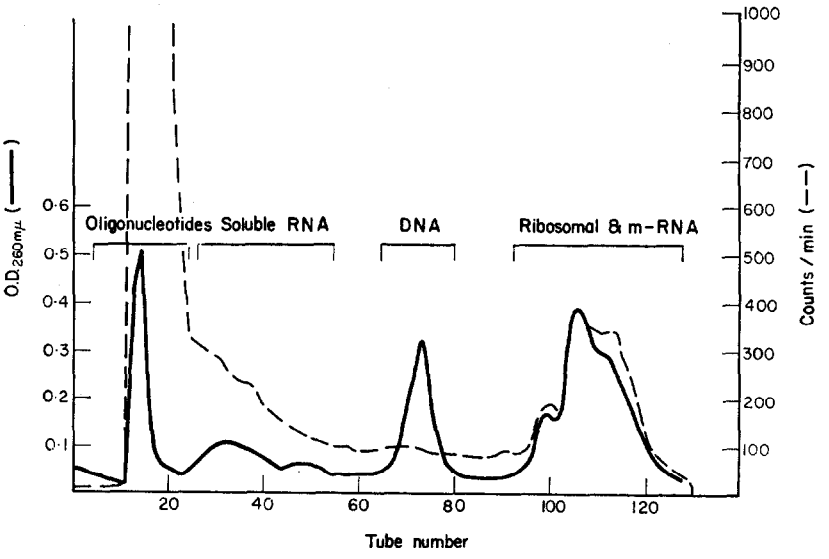

FIG. 4. METHYLATED ALBUMIN KIESELGUHR SEPARATION OF NUCLEIC ACIDS OF PEANUT AXES AFTER 24 hr GERMINATION.

See Experimental for procedures.

Further, there was some asymmetrical ^{32}P labeling on the trailing shoulder of the heavy ribosomal peak. In germinating peanut cotyledons, such a region acts as template in an *in vitro* amino acid incorporating system.[18] The presence of the presumed m-RNA synthesis in peanut axes at 30 hr (24 hr germination plus 6 hr incubation) corresponds to the initiation of synthesis of new m-RNA species by wheat embryos[19] and to the observation[20] that peanut axes are actively synthesizing protein at 24 hr. It has not been possible to ascertain if synthesis occurs any earlier than 24 hr in peanut axes through the use of methylated albumin chromatography because of difficulties with penetration and incorporation of ^{32}P.

Thus at 24 hr ^{32}P was not incorporated into DNA although some ribosomal RNA and presumed m-RNA synthesis occurred (Fig. 4). As previously indicated, this period was

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prior to the appearance of the pyrimidine deoxynucleoside kinases (Fig. 2) but corresponded to the time when uridine kinase activity began (24 hr germination plus 6 hr incubation in ^{32}P).

Figure 5 represents the labeling of nucleic acid fractions from peanut seedlings after 54 hr germination. The labeling patterns as well as the specific activities of the various fractions at 72 hr were the same as those at 54 hr. For this reason, the 72 hr labeling profile is not

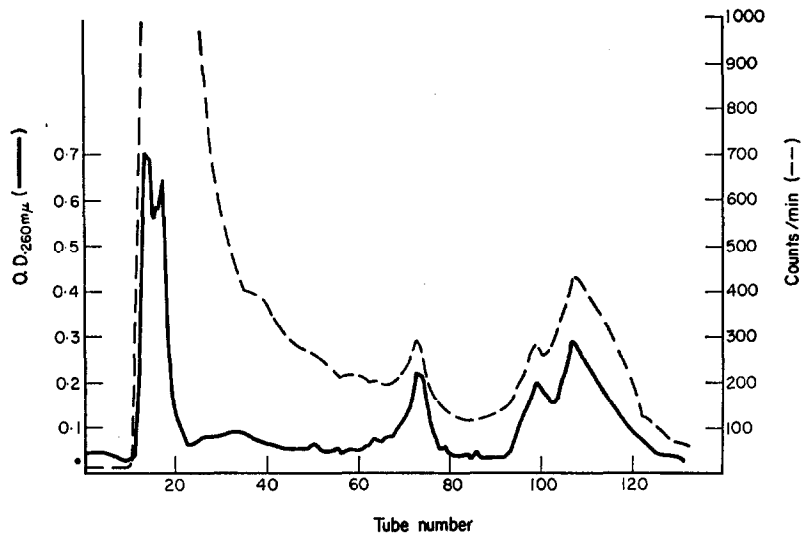

FIG. 5. METHYLATED KIESELGUHR SEPARATION OF NUCLEIC ACIDS OF PEANUT AXES AFTER 54 hr GERMINATION.

See Experimental for procedures.

shown. All of the kinases were presumably present at 54 and 72 hr (Figs. 1 and 2); and indeed, all nucleic acid fractions, including DNA, were labeled (Fig. 5). According to the data presented in Figs. 1 and 2, relatively higher pyrimidine nucleoside kinase activities were displayed as germination proceeded. These activities were seemingly confirmed by the higher specific activities (CPM/unit absorbance) of the RNA fractions (Fig. 5). The synthesis or activation of the pyrimidine deoxynucleoside kinases (Fig. 2) preceded subsequent DNA synthesis (Figs. 4 and 5). Therefore, active incorporation of DNA precursors such as thymidine (Table 1) and ^{32}P (Figs. 4 and 5) closely paralleled the appearance of the pyrimidine deoxynucleoside kinases. Utilization of these components for DNA synthesis prior to the appearance of the enzymes was nominal. These results suggest that, similar to other systems,[1,5–11,21,22] pyrimidine deoxynucleoside kinases are extremely important for the synthesis of DNA during the early development of peanut axes.

EXPERIMENTAL

Germination Procedure

Peanuts (*Arachis hypogaea* var. N.C.2) were planted as described previously.[14] The thoroughly moistened scrolls were allowed to incubate at 25° in the dark for various times up to 96 hr. The scrolls were kept moist by frequent waterings throughout the incubation period.

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Nucleoside Kinase Assays

The various pyrimidine nucleoside kinase activities were assayed as described in the previous report.[14]

Protein Determination

Protein content of the various samples was determined on TCA precipitates.[23] Standard curves were determined using bovine serum albumin.

Total Nucleic Acid Determinations

From peanuts planted and incubated as described above, axes (25) were harvested every 12 hr for 96 hr. The samples were immediately frozen and stored until all were collected. Nucleic acids were extracted by the method of Smillie and Krotokov[24] as modified by Nitsan and Lang.[17] Ribonucleic acid was estimated as phosphorus[25] and deoxyribonucleic acid content by the diphenylamine reaction.[26]

Thymidine Incorporation

Peanut seedlings in various stages of development (24, 42 and 60 hr) were incubated with constant stirring at room temp. (25°) in deionized water containing ^{14}C-thymidine (10 μc) (48 mc/mmole) for 4 hr. The axes were surface-sterilized in NaOCl prior to and after incubation, rinsed twice in 10^{-3} M non-isotopic thymidine, twice with distilled water and then fractionated into cold perchloric acid (PCA) soluble and insoluble fractions.[16] The extent of radioactive incorporation of ^{14}C-thymidine into the respective fractions was determined by standard scintillation counting techniques.

^{32}P Incorporation into Nucleic Acids

Seedlings were harvested at 24, 54 and 72 hr. The axes were surface sterilized with NaOCl and incubated at room temp. with constant stirring in 10^{-4}M citrate buffer (pH 6·0) and 500 μc carrier-free ^{32}P for 6 hr. The axes were then thoroughly rinsed with distilled water, 0·5 M sodium–potassium phosphate buffer (pH 7·0) and distilled water, respectively. Nucleic acids that were used for methylated albumin chromatography were isolated and separated according to the procedure of Ingle *et al.*[27] Methylated albumin kieselguhr columns were prepared at double the formulation of Mandell and Hershey.[28] 4 ml fractions were collected and absorbance was determined at 260 nm. An aliquot (1·0 ml) of each fraction was plated onto a planchet for radioactivity assay in a Nuclear-Chicago Geiger–Mueller counter equipped with a micromil window.

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