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ASSOCIATION OF THYMIDINE AND URIDINE KINASE ACTIVITIES WITH CHANGES IN NUCLEIC ACID LEVELS DURING PEANUT FRUIT ONTOGENY*

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Abstract—Various stages of pegs, cotyledons and embryonic axes from maturing peanut fruits were examined for their ability to phosphorylate thymidine and uridine. Highest specific activities during peg elongation were found just prior to increases in endosperm nuclei and embryo cell numbers. In the developing cotyledons and axes, the net kinase activities of crude extracts reached a maximum 1–2 weeks before maximal RNA and DNA contents were attained. An exception was the apparent lack of any relationship between uridine kinase activities and RNA levels in developing embryonic axes. The present results support the observation that peanut axes are devoid of thymidine and uridine kinases during the first 24 hr of germination, as fully developed fruits had very low specific activities for both of these phosphate transferases.

INTRODUCTION

THYMIDINE kinase (ATP:thymidine 5'-phosphotransferase, E.C. 2.7.1.21) has been reported to occur in a large number of organisms[1–3] and tissue types.[4,5] In some bacterial systems, thymidine kinase appears to function as a scavenger enzyme[6] through phosphorylation of thymidine released during DNA degradation. However, in higher organisms, activity of this enzyme is more closely associated with DNA synthesis[2,7] and cell proliferation.[4,5,8]

In plants, for example, Hotta and Stern [7] found the transient appearance of thymidine kinase activity in maturing lily microspores just prior to an increase in DNA content of the cells. Wanka and Walboomers[9] reported a correlation between thymidine kinase levels and changes in DNA content during the germination of maize seeds. Similar relationships between both thymidine and uridine kinase levels and the corresponding changes in DNA and RNA content appear to exist during the early germination phase of peanut seedlings.[10] Schwarz and Fites[10] further reported that the appearance of thymidine kinase activity was associated with an enhanced incorporation of DNA precursors.

During germination of both corn[9] and peanut[10] seeds, thymidine kinase activity was not present until 36 hr after imbibition; whereas, uridine kinase appeared 12 and 24 hr after germination in corn[9] and peanuts,[10] respectively. Thus, it was of interest to us to ascertain

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[1] F. WANKA, I. K. VASIL and H. STERN, *Biochem. Biophys. Acta* **85**, 50 (1964).
[2] A. KORNBERG, I. R. LEHMAN, M. J. BESSMAN and E. S. SIMMS, *Biochem. Biophys. Acta* **21**, 197 (1956).
[3] S. M. WEISSMAN, R. M. S. SMELLIE and J. PAUL, *Biochem. Biophys. Acta* **45**, 101 (1960).
[4] R. E. BELTZ, *Arch. Biochem. Biophys.* **99**, 304 (1962).
[5] E. BRESNICK and V. B. THOMPSON, *J. Biol. Chem.* **240**, 3967 (1965).
[6] R. L. BLAKLEY and E. VITOLS, *Ann. Rev. Biochem.* **37**, 201 (1968).
[7] Y. HOTTA and H. STERN, *Proc. Natl. Acad. Sci. U.S.* **49**, 861 (1963).
[8] F. J. BOLLUM and V. R. POTTER, *J. Biol. Chem.* **233**, 478 (1958).
[9] F. WANKA and J. M. WALBOOMERS, *Z. Pflanzenphysiol.* **55**, 458 (1966).
[10] O. J. SCHWARZ and R. C. FITES, *Phytochem.* **9**, 1899 (1970).

whether the above pyrimidine nucleoside and deoxynucleoside kinases were present and played a physiological role during seed development and maturation or whether these enzymes remained absent until the 12–40 hr period of seed germination. During peanut seed ontogeny the former occurs, and is the subject of this report.

RESULTS

Uridine kinase, thymidine kinase, and phosphatase activities were determined during the very early period of peanut fruit development by assaying for these enzymes at three successive stages of 'peg' development (Table 1). These stages essentially conform to those

TABLE 1. SPECIFIC ACTIVITIES OF PHOSPHATE TRANSFERASES AND PHOSPHATASE AT THREE SUCCESSIVE STAGES OF PEG DEVELOPMENT

Developmental stage*	Specific activity (% phosphorylation or dephosphorylation/min/mg protein		
	Uridine kinase	Thymidine kinase	Phosphatase
P_1	8·6	9·0	254·4
P_2	12·3	28·5	166·9
P_3	14·5	37·3	84·2

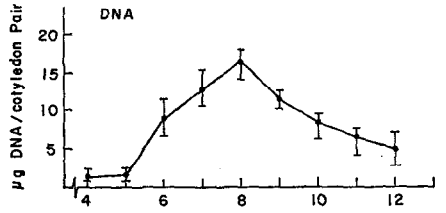
* P_1 were aerial pegs, dark green at rigid and pointed tip. P_2 stage were underground pegs, white to light green at tip (still pointed). P_3 were white fleshy underground pegs at the onset of bulb formation.

described by Smith[11] (Fig. 20, b–d). During this time, peg elongation proceeds very rapidly[12] while fruit development is slow until after the P_3 stage (Table 1). Although there is little mitotic activity during this period of most rapid peg elongation,[12] the terminal 1·0 cm of peg tissue, which contains an intercalary meristem in addition to the ovules, was nevertheless characterized by increasing specific activities of thymidine and uridine kinases and a rapidly declining amount of phosphatase activity (Table 1). Although thymidylate was used as substrate for the phosphatase assay (see Experimental), this enzyme is non-specific and capable of dephosphorylating a number of nucleotides and deoxynucleotides.[15] Further, since this phosphatase affects the rate of thymidine phosphorylation,[15] the increases in kinases recorded in Table 1 may be partially attributable to the decreasing rate of the back reaction (phosphatase).

Developmental seed stages (Figs. 1–5) are based on the number of weeks after the pegs turned at right angles in the soil (stage P_3, Table 1) and were ascertained for assay purposes according to the description of Pattee *et al.*[13]

In the cotyledons, the DNA content reached a maximum at week 8 and then declined during the remainder of maturation (Fig. 1); similar results for developing peanut cotyledons have previously been reported.[14] Net thymidine kinase activity reached a peak at week 7, one week prior to maximum DNA content. During late maturation, net thymidine kinase

[11] B. W. SMITH, *Am. J. Bot.* **37**, 808 (1950).
[12] B. W. SMITH, *Am. J. Bot.* **43**, 233 (1956).
[13] H. E. PATTEE, J. A. SINGLETON, E. B. JOHNS and B. C. MULLIN, *J. Agric. Food Chem.* **18**, 353 (1970).
[14] A. B. ALDANA, R. C. FITES and H. E. PATTEE, *Plant Physiol.* (submitted).



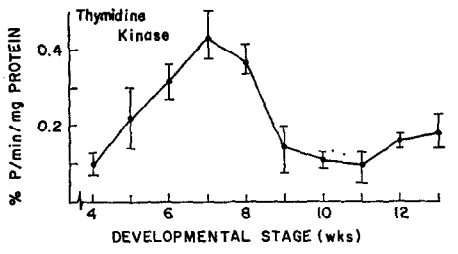
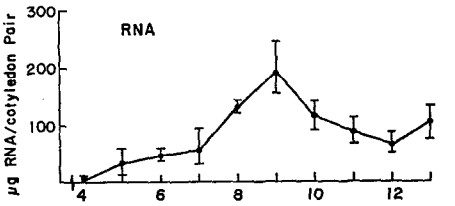


FIG. 1.



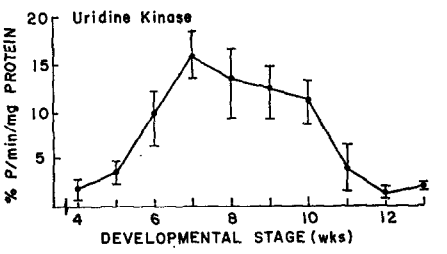
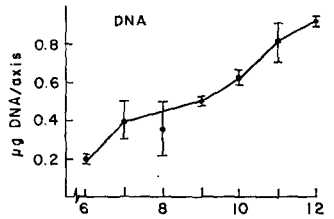


FIG. 2.

FIG. 1. SPECIFIC THYMIDINE KINASE ACTIVITY PROFILES AND DNA LEVELS IN DEVELOPING PEANUT COTYLEDONS.
Vertical bars represent absolute deviations from the mean.

FIG. 2. SPECIFIC URIDINE KINASE ACTIVITY PROFILES AND RNA LEVELS IN DEVELOPING PEANUT COTYLEDONS.
Vertical bars as in Fig. 1.



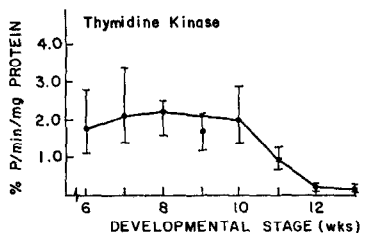
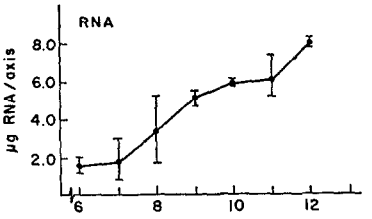


FIG. 3.



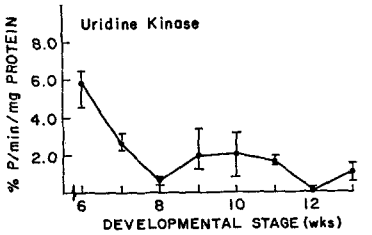


FIG. 4.

FIG. 3. SPECIFIC THYMIDINE KINASE ACTIVITY PROFILES AND DNA LEVELS IN DEVELOPING EMBRYONIC AXES.
Vertical bars as in Fig. 1.

FIG. 4. SPECIFIC URIDINE KINASE ACTIVITY PROFILES AND RNA LEVELS IN DEVELOPING EMBRYONIC AXES.
Vertical bars as in Fig. 1.

activities increased somewhat but without any subsequent corresponding change in DNA levels.

A similar pattern emerged between RNA levels and uridine kinase activities during development of the cotyledons (Fig. 2). Here, RNA content reached a peak at week 9 preceded by a maximum in uridine kinase activity at 7 weeks. Although absolute amounts of RNA are different (due in part to differences in analytical procedures and the methed of determining developmental stage), the increase in RNA content of the cotyledons after 12 weeks (Fig. 2) has been noted previously.[14]

Embryonic axes of developing peanut seeds were not large enough to be easily separable from the cotyledons until the 6 week stage (Figs. 3 and 4). DNA in the axes increased throughout maturation (Fig. 3). Thymidine kinase activity was nearly maximal by the time

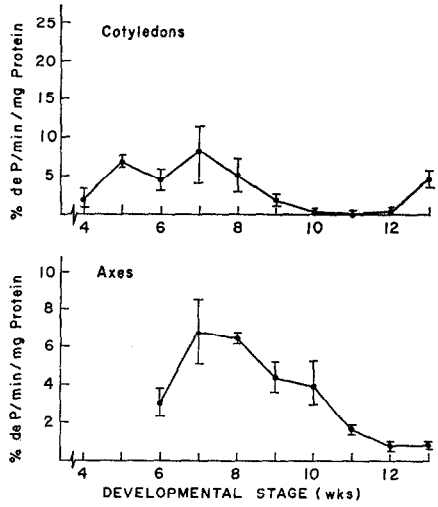


FIG. 5. SPECIFIC ACTIVITY PROFILES OF PHOSPHATASE IN DEVELOPING PEANUT AXES AND COTYLEDONS. Vertical bars as in Fig. 1.

axes could be successfully dissected from the cotyledons (Fig. 3), and remained high until after the 10th week of maturation. The phasing out of thymidine kinase activity during late maturation is consistent with the observation that germinating peanut axes are devoid of any detectable thymidine kinase activity until after 36 hr germination.[10]

The highest levels of net uridine kinase activity in developing peanut axes were apparently attained (Fig. 4, week 6) long before maximum RNA content (Fig. 4, week 12). In general, then, there did not appear to be any relationship between RNA content and uridine kinase activities during maturation of peanut axes.

The ability of extracts from developing peanut cotyledons and axes to dephosphorylate thymidine 5′-monophosphate was followed during maturation. As shown in Fig. 5, phosphatase activity followed a similar pattern in developing cotyledons and axes. As indicated earlier, the presence of such phosphatase activity affects absolute rates of thymidine and uridine phosphorylation[15] so all of the kinase activities presented herein should thus be viewed as net rather than absolute.

[15] O. J. SCHWARZ and R. C. FITES, *Phytochem.* **9**, 1405 (1970).

DISCUSSION AND CONCLUSIONS

The highest specific activities for thymidine kinase were found in peg tissues (Table 1). Enzymic levels were particularly high during the period corresponding to minimal mitotic activity in ovules[12] but very rapid peg growth. Such growth appears to occur by means of elongation of cells formed in an intercalary meristem located approximately three millimeters from the peg tip.[12]

The question arises as to the physiological purpose of the enhanced kinase levels observed during the P_2 and P_3 stages of fruit development (Table 1). During peanut seed germination, uridine and thymidine kinase activities closely parallel phosphorylation, respectively, of other pyrimidine nucleosides and deoxynucleosides.[10] Do the present activities (Table 1), thus, reflect increasing phosphorylation of pyrimidine RNA and DNA precursors necessary for the concomitant peg growth at this time or for utilization in subsequent events of peanut fruit and seed development? For the following reasons, the latter would seem to be a more appropriate conclusion. At the P_3 stage, pegs have already completed their most rapid rate of elongation[12] while specific activities at P_3 are even higher than at P_2 (Table 1). In addition to the present observations (Figs. 1–3), previous studies[7,9,10] have indicated that thymidine and uridine kinase levels increase or reach a peak prior to corresponding changes in DNA or RNA content, respectively. Thus, it does not seem likely that phosphorylation of precursors for RNA and DNA involved in peg growth would be rising at a time of diminishing elongation rate.[12]

On the other hand, the increased specific activities of thymidine and uridine kinases (Table 1) preceded by 7–10 days an exponential rise in the number of endosperm nuclei and embryo cells.[12] Further growth after the P_3 stage is largely in the form of pod enlargement and is initiated slightly in advance of renewed endosperm and embryo development.[12] Hence, it seems more appropriate to associate the enhanced thymidine and uridine phosphorylation recorded in Table 1 with these events rather than peg elongation *per se*.

After an interval of approximately 4 weeks, specific activities as well as relative activities (data not shown) for kinases localized in the embryo (Figs. 1 and 2, weeks 4 and 5) proved to be lower than those found in fruit tissue as a whole (Table 1). With the exception of uridine kinase from embryonic axes (Fig. 4), an association was found to exist between the pyrimidine kinases and subsequent changes in macromolecular content of RNA and DNA (Figs. 1–3) in the developing tissues of the seed. The highest phosphorylation rate of uridine or thymidine preceded by 1–2 weeks the respective peaks in RNA or DNA levels.

As indicated earlier, the major objective of this investigation was to determine if pyrimidine nucleoside and deoxynucleoside kinases were present during seed development and maturation. As discussed above, these enzymes of DNA and RNA precursor phosphorylation appear to be associated with the biochemical and physiological events leading to changes in DNA and RNA content during fruit development and seed maturation. An important question which remains is why uridine and thymidine kinase activities are phased out during late maturation of the embryonic axes (Figs. 3 and 4) only to reappear in the same tissues some 24–36 hr after germination is initiated.[10]

EXPERIMENTAL

Assay procedure. Enzyme assays for uridine kinase and thymidine kinase have been previously described;[15] phosphatase was similarly assayed with the substitution of thymidine monophosphate for thymidine.[15] The reaction mixtures were incubated for 10, 15, or 20 min at 30–31°. The reaction was terminated by the addition of 300 μl of cold MeOH and rapid cooling to 4° in an ice bath followed by centrifugation at

15000 *g* for 15 min. The supernatant was spotted on Whatman No. 3 MM paper and chromatographed in *iso* PrOH, H_2O, NH_4OH (65·4:11·5:1) as solvent. Developed chromatograms were air-dried before detecting the nucleosides and nucleotides with short-wave UV light. These fractions were cut from the chromatograms and counted in a liquid scintillation counter. Specific activity was determined as the per cent nucleoside phophorylated (or nucleotide dephosphorylated) per min per mg of protein (100% phosphorylation equivalent to formation of 10 μmole nucleotide). All specific activity values were the average of 4–8 replications.

Enzyme source. Immature peanut seeds (variety NC-2) were obtained from field grown plants. Initial stages of developing pegs (terminal 1·0 cm) used as enzyme sources (P_1, P_2, and P_3) were similar to those described by Smith.[11] Peanut seed development was followed from 4 weeks after the pegs turned at right angles through 13 weeks.[13]

Preparation of crude enzyme extract. Due to the small size of embryonic axes, peanut seeds were not separated into axes and cotyledons until the week 6 stage of development.[13] Crude homogenates were prepared in cold (0–4°) 0·2 M potassium phosphate buffer, pH 7·0, by grinding 25 cotyledonary pairs or 25 axes (per replication) in a mortar and pestle. The resulting brei was passed through several layers of fine-mesh nylon cloth and centrifuged in the cold at 32000 *g* for 30 min. The resulting supernatant was used as the source of crude enzyme.

Determination of protein and total nucleic acids. Protein and total nucleic acid levels were determined as previously reported.[10] Two to five replicates were used for RNA or DNA determinations at each developmental stage.

Key Word Index—Arachis hypogaea; Leguminosae; peanut; thymidine kinase; uridine kinase; nucleic acid.