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THYMIDINE KINASE FROM PEANUT SEEDLINGS*

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Abstract—Thymidine kinase (ATP:thymidine 5′-phosphotransferase, EC 2.7.1.21) from the hypocotyls of germinating peanuts (*Arachis hypogaea* var. N.C.2) required the presence of a divalent cation and a phosphate donor (ATP) for the production of thymidine 5′-monophosphate. The divalent cation requirement could be fulfilled by Mg^{2+}, Mn^{2+}, Ca^{2+}, Cu^{2+} and Zn^{2+}. Thymidylate phosphatase activity was present in all of the enzyme preparations despite attempts to inhibit this action. The kinase was relatively heat stable up to 60°; further temperature elevation resulted in complete inactivation. The production of TMP was optimal over a pH range of 7·5–9·0. The rate of thymidine phosphorylation was affected by uridine, deoxyuridine, cytidine and deoxycytidine. Partially purified extracts (15–20-fold) of thymidine kinase also phosphorylated the above pyrimidine nucleosides and deoxynucleosides.

INTRODUCTION

THE PHOSPHORYLATION of thymidine (dT) to thymidine monophosphate (TMP) is catalyzed by thymidine kinase in a wide variety of organisms[1–4] and tissue types.[5–14] In plants, however, this enzyme has been investigated in only a few species. Hotta and Stern[15] found a marked periodicity of thymidine kinase activity in developing microspores of *Lilium*, thereby implicating the participation of thymidine kinase in the phosphorylation of thymidine for its subsequent incorporation into DNA. Wanka[16] reported that thymidine kinase activity did not appear in corn seedlings until 36 hr after germination. It would thus appear that the thymidine kinase of plants plays an important physiological role during development rather than functioning as a scavenger enzyme, as suggested for some bacteria.[17,18] With the

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exception of the enzyme in corn,[2] there is little information pertaining to the physical characteristics and properties of thymidine kinase isolated from plants. In this and a later report, we have attempted to describe some of the properties of this enzyme and its role in the dT phosphorylating system of peanut seedlings.

RESULTS

In order to determine a suitable source of enzyme, germinating peanut seedlings (5 days after planting) were separated into various organs and tissues. Thymidine kinase activity was detected in all tissues of such seedling peanuts. Although crude homogenates from hypocotyl and primary root tissues of the germinating peanut had the highest and equivalent phosphorylating activity (specific activities of 0·38 and 0·36, respectively), hypocotyl tissue was chosen for subsequent work because of its relative ease of homogenization and low polyphenol oxidase content.

TABLE 1. REQUIREMENTS FOR THYMIDINE KINASE ACTIVITY USING AN ACETONE POWDER EXTRACT OF 5-day-old PEANUT HYPOCOTYLS

Components other than thymidine	Activity (% thymidine phosphorylated/min)	Activity as % of that of complete mixture
ATP, Mg^{2+}, enz.	1·81	100
Enz.	0·02	1·1
Mg^{2+}, enz.	0	0
ATP, enz.	0·95	52·5
ATP, EDTA, enz.	0·15	8·29
ATP, Mg^{2+}	0	0

Acetone powders were extracted with 0·2 M phosphate buffer (pH 7·0) and used as the enzyme source. EDTA was added to a final concentration of 1×10^{-3} M. Thymidine was always present; other assay components were added in volumes and concentrations as described in Assay Procedure (see Experimental). Total volume of assay mixture was 140 μl. Assay time was 10 min.

Various requirements for the phosphorylation of thymidine by thymidine kinase are shown in Table 1. As indicated, adenosine triphosphate (ATP) and Mg^{2+} were required for the phosphorylation of dT. Bresnick and Thompson,[7] using partially purified thymidine kinase from Novikoff hepatoma, MDAB hepatoma and Walker carcinosarcoma, reported a similar requirement for ATP and Mg^{2+}; Okazaki and Kornberg[19] showed that ATP and deoxyguanosine triphosphate (dGTP) could serve as phosphate donors in enzyme isolated from *Escherichia coli*. A small amount of phosphorylation was occasionally observed in the absence of exogenous ATP. This was probably due to the presence of low concentrations of endogenous phosphate donors. These preparations also contained some residual metal ion contamination as suggested by the fact that the addition of ethylenediaminetetraacetate (EDTA) to such preparations effectively reduced activity (Table 1). When Mg^{2+} was added in excess of EDTA, activity was restored. Okazaki and Kornberg[20] found that the addition of bovine serum albumin (BSA) was necessary to attain maximum reaction rates in preparations of thymidine kinase from *E. coli*. They concluded that the BSA had a stabilizing effect

[19] R. OKAZAKI and A. KORNBERG, *J. Biol. Chem.* **239**, 275 (1964).
[20] R. OKAZAKI and A. KORNBERG, *J. Biol. Chem.* **239**, 269 (1964).

on the enzyme. However, no observable effects were noted when BSA (0·2 mg/ml and 1·0 mg/ml) was added to peanut kinase assay mixtures.

The following procedure was developed to identify the reaction products of the enzyme assay. Using ^{14}C-dT as substrate and a crude enzyme preparation from 5-day-old etiolated peanut hypocotyls, complete reaction mixtures (incubated for 30 min) and controls containing heat inactivated enzyme were chromatographed on thin-layer plates of polyethyleneimine (PEI) cellulose.[21] Chromatograms were developed first in distilled water, allowing the solvent front to traverse the entire chromatogram. The chromatograms were then air-dried and, except for the origin, u.v.-absorbing spots were marked. Phosphorylated compounds were then separated from the origin by chromatography in 1 M LiCl, allowing the solvent front to migrate to just below the lowest marked spot.

With ^{14}C-dT as substrate, radioactivity was found only in spots corresponding to dT and TMP. No TMP was detectable in boiled controls. The TMP from several PEI-cellulose chromatograms was pooled and chromatographed on Whatman 3 MM and on PEI-cellulose in n-BuOH–HOAc–H_2O (2:1:1) and H_2O saturated *n*-BuOH, respectively. In each case only one spot, corresponding to authentic TMP, was detected. Furthermore, the isolated product was identical to TMP in u.v. maxima and in commonly used spectral ratios. Presence of the deoxyribose moiety was confirmed by the method of Brody.[22] Thymidine 5′-diphosphate (TDP) and thymidine 5′-triphosphate (TTP) were never detected in any of our preparations.

Contrary to other reports[1,23] and despite the high concentration of phosphate buffer (0·2 M) used in the reaction mixture, our preparations contained active phosphatases that were able to remove all of the phosphate from ATP and convert TMP to dT. Wanka and Walboomers[24] noted a sharp decrease in reaction velocity of product formation after 42 per cent of the original substrate was converted to TMP and speculated that this deviation was due to the lowering of ATP concentration by the dephosphorylation which they had observed. Peanut preparations behaved in a similar fashion (Fig. 1). The data closely fit the curves presented by Wanka and Walboomers[24] for first-order reaction kinetics of dT phosphorylation in corn. Assuming a first-order reaction, the initial velocity (V_0) for the phosphorylation of dT was therefore calculated according to $V_0 = (X_0/t)(\ln X_0/X)$, where X_0 and X are nucleoside concentrations initially and after time (t), respectively.[24] A plot of $\log X/X_0$ vs. t yielded a straight line for values up to 35 per cent phosphorylation, indicating first-order kinetics for that range.[25] The ATP concentration was present in saturating amounts during this phase of the reaction (up to 35 per cent phosphorylation). The flattening of the reaction rate curve above 35 per cent phosphorylation (Fig. 1) was due to the degradation of ATP by phosphatase action. Addition of extra enzyme to an assay preparation that had already phosphorylated 40 per cent of its substrate brought no enhancement of phosphorylation rate while addition of ATP (5×10^{-2} M; 20 μl) restored the reaction to its original rate. Therefore, we concluded that enzyme inactivation under our assay conditions probably did not contribute to the sharp loss of phosphorylating ability shown in the latter portion of the curve (Fig. 1). Subsequent assays were adjusted to give a maximum of 25 per cent phosphorylation during the incubation time in order to minimize errors which might have occurred because of the aforementioned deviations. Fluoride (10^{-3} M) was added to

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[22] S. BRODY, *Acta Chem. Scand.* **7**, 502 (1953).
[23] F. WANKA and F. W. BAUER, *Z. Pflanzenphysiol.* **58**, 175 (1967).
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[25] J. B. NEILANDS and P. K. STUMPF, *Outlines of Enzyme Chemistry*, p. 92, John Wiley, New York (1964).

the assay mixture in an attempt to inhibit the deoxynucleoside phosphatase activity[5] but no increase in dT phosphorylation was observed. In a further attempt to ascertain whether or not the dephosphorylation of TMP had any effect on the initial reaction rates, non-isotopic TMP was added in varying concentrations to the assay. This was done in an attempt to dilute the labelled product and in effect flood the reverse reaction with non-isotopic TMP. An increase in the initial velocity of dT phosphorylation was observed, indicating that the rates of dT phosphorylation recorded were indeed influenced by the presence of phosphatase activity. Under these conditions comparisons of enzyme activities at different stages of development are difficult at best, for there is always the possibility of fluctuations in the

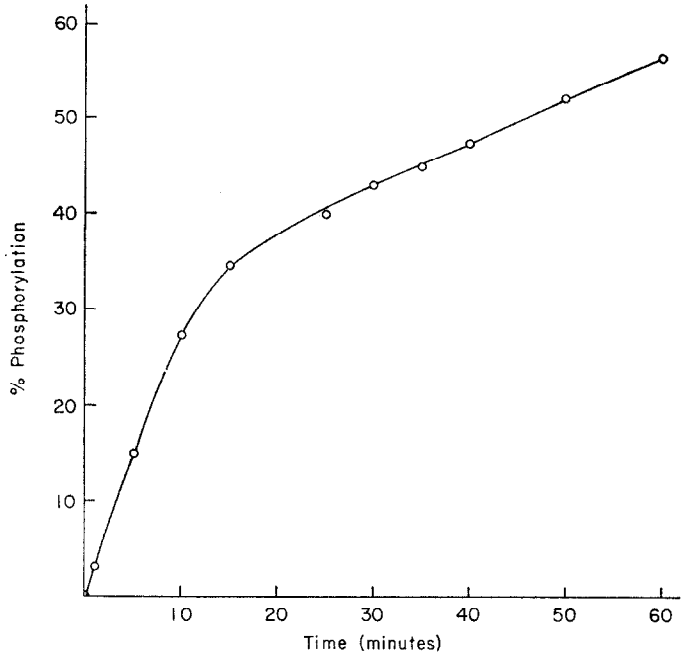

FIG. 1. TIME COURSE OF THYMIDINE PHOSPHORYLATION BY A CRUDE HOMOGENATE OF 5-day-old PEANUT HYPOCOTYLS.

The enzyme extract was prepared from 5-day-old peanut hypocotyls. Each point represents an average of eight replicates. The average deviation from the mean for the points was ±0·85.

levels of the competing reaction or reactions. Beltz[5] suggested that for rat liver the rate of dephosphorylation of TMP at initial velocities of TMP formation posed no problem. He speculated that the degree of saturation of the phosphatase was not sufficiently high (at the low TMP concentrations present during the initial stages of the reaction) to catalyze hydrolysis of the phosphate esters at a significant rate. Since this is apparently not the case in germinating peanuts, the rates presented here should be viewed as net rather than absolute.

Wanka *et al.*[2] heated crude extracts of corn as an initial step in their purification procedures for thymidine kinase. Thus, the heat stability of the peanut kinase enzyme was determined for possible use in purification. Aliquots of a crude enzyme preparation were incubated for 15 min at temperatures varying from 20° to 70° and cooled rapidly in an ice bath. Precipitated protein was removed by centrifugation before assaying supernatants (Fig. 2). The enzyme was stable up to 40° and then rapidly lost activity, being completely

inactivated after heating to 70° for 15 min. As indicated, specific activity of the preparations failed to increase. Under similar conditions with crude enzyme extracts from corn, Wanka[16] reported that enzyme activity was stable to 50°, but was completely inactivated at 60°.

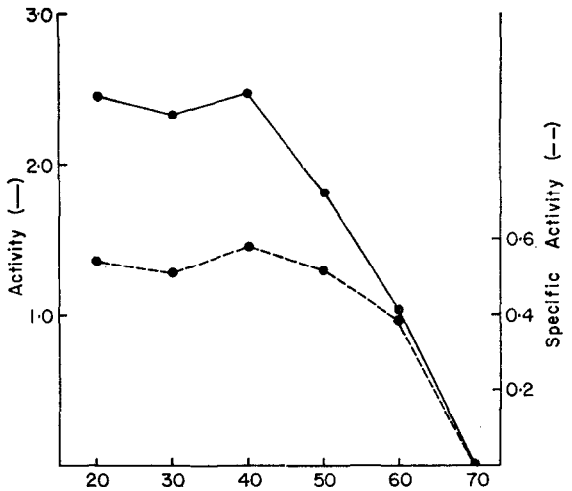

FIG. 2. HEAT SENSITIVITY OF THYMIDINE KINASE FROM PEANUT HYPOCOTYLS.

Aliquots of a crude enzyme preparation from hypocotyls of 5-day-old peanut seedlings were incubated for 15 min at the indicated temperatures and rapidly cooled in an ice bath. The aliquots were then centrifuged for 10 min at 30,000 *g* and assayed.

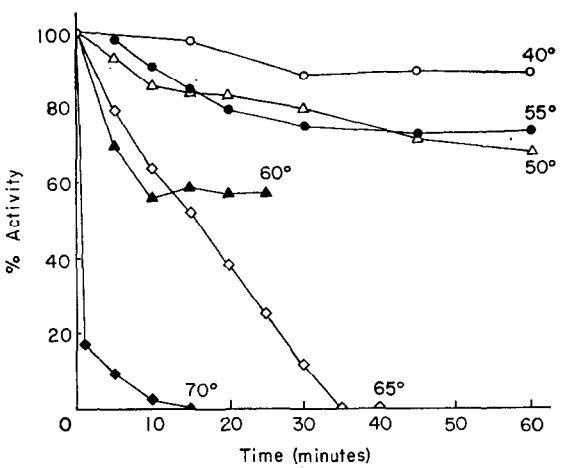

FIG. 3. TIME COURSE OF HEAT INACTIVATION OF THYMIDINE KINASE.

Crude enzyme preparations were subjected to various incubation times at constant temperatures after cooling to 4°; the preparations were centrifuged for 10 min at 30,000 *g* and assayed.

The possibility of a longer heat treatment for purification purposes was investigated. The crude preparations were subjected to varying incubation times at constant temperatures (Fig. 3). The kinase was very stable at 40° losing about 10 per cent of its activity in 30 min, then remaining stable for as long as 4 hr (data not shown). Stability at 50° and 55° was essentially the same showing slightly greater initial activity loss than at 40°. A rapid loss in

activity of about 40 per cent was observed at 60° in the first 10 min of incubation; further incubation showed little additional change. The initial activity loss could have resulted from a partial disruption of the enzyme's tertiary structure rendering it less efficient in phosphorylating ability. A sharp break of this type could also indicate the presence of more than one kinase. This is possible if the heat stabilities of the enzymes differ greatly. Further elevation of temperature (Fig. 3) produced progressively greater losses of activity. At no time was there a significant increase in the specific activity of these preparations.

Since heat inactivation was ineffective, the following procedure was used to purify peanut thymidine kinase. Acetone powders were extracted with phosphate buffer, 0·2 M, pH 7·0 (0–4°), for 20 min in an ice bath (40 ml buffer/g acetone powder). The suspension was then passed through several layers of fine-mesh nylon cloth and centrifuged in the cold at 32,000 *g* for 20 min. Ammonium sulfate was added to the supernatant to 50 per cent saturation, after which the preparation was stirred 20 min in an ice bath. Following centrifugation as above, the resulting supernatant was brought to 80 per cent saturation and again stirred in an ice bath for 20 min. After a final centrifugation, the supernatant was discarded. The pellet was resuspended in cold phosphate buffer (0·2 M, pH 7·0) and dialyzed against

TABLE 2. PURIFICATION OF THYMIDINE KINASE FROM THE HYPOCOTYLS OF GERMINATING PEANUT SEEDLINGS

Fraction	Specific activity*	Protein (mg)	Total activity†	Per cent recovery
1. Extract of acetone powder	0·95	116	110	100
2. $(NH_4)_2SO_4$ (50–80% cut)	2·67	15	40·1	36·5
3. Sephadex G-100 fraction	16·2	1·8	29·2	26·5

* Specific activity (% phosphorylation/min/mg protein).
† Total activity (specific activity × mg total protein).

two changes of the same buffer for 8–12 hr. The preparation was then centrifuged to remove a precipitate formed during dialysis. The soluble material was chromatographed on a Sephadex G-100 (2·5 × 40 cm) column previously equilibrated with phosphate buffer, 0·2 M, pH 7·0. With a fraction size of 3 ml, the enzyme was consistently recovered in fraction 31–38 as a symmetrical peak, which corresponded to a molecular weight of approximately 50,000.

This procedure routinely resulted in a 15–20-fold purification of the enzyme (Table 2). However, compared to the crude extracts prepared directly in phosphate buffer, the purified extract (Fraction 3) represented a 45-fold purification. Attempts to further purify the Sephadex G-100 fraction were unsuccessful. This fraction was applied to DEAE Sephadex and DEAE Cellulose columns with only 10 per cent recovery and no further increase in specific activity.

Wanka *et al.*[2] have shown that thymidine kinase from corn and wheat is dissociable into two subunits by $(NH_4)_2SO_4$ fractionation. The fractionation methods described for the isolation of corn and wheat subunits and several variations of these methods were applied to crude preparations derived from acetone powders of peanut seedlings. However, we were not able to demonstrate the presence of subunits for the peanut kinase by such techniques.

The effect of pH on dT phosphorylation was measured over a range from pH 6·0–9·5 using phosphate buffer from pH 6·0–8·0 and Tris at higher pHs. Inorganic phosphate concentrations were maintained at 0·2 M, regardless of buffer or pH. The optimal pH

range for the phosphorylation of dT was quite broad (pH 7·5–9·0). Although information concerning plant systems is lacking, the optimal pH range for a number of other kinases[26,27] varies between pH 6·0–9·0.

The ability of peanut thymidine kinase to phosphorylate dT was tested in the presence of some common divalent cations. The cations were added to the complete assay mixture (including Mg^{2+}) as their chloride salts at 10^{-3} M. The enzyme was remarkably stable in the presence of all the cations tested (Zn^{2+}, Cu^{2+}, Ca^{2+}, Mn^{2+}). There was no observable enhancement or inhibition of the kinase activity under these conditions. Data are lacking on the metal ion stability of thymidine kinase from other plant sources; however, enzyme derived from Walker carcinosarcoma[7] was found to be inhibited by the addition of Mn^{2+}, Co^{2+}, Ca^{2+}, Ni^{2+}, Sr^{2+}, Fe^{2+}, Ba^{2+}, or Zn^{2+} in the presence of Mg^{2+} ions.

The ability of some of the above divalent ions to substitute for the Mg^{2+} requirement is shown in Table 3. Zn^{2+}, Cu^{2+} and, to some extent, Mn^{2+} and Ca^{2+} sustained dT phosphorylation when substituted for Mg^{2+} in the assay mixture.

TABLE 3. EFFECT OF DIVALENT CATIONS ON THYMIDINE PHOSPHORYLATION

Addition	Activity (% phosphorylation/min)
Control (EDTA)	0·15
$MgCl_2$	1·70
$ZnCl_2$	1·71
$CuCl_2$	1·51
$CaCl_2$	1·10
$MnCl_2$	1·27

Reaction mixture consisted of 50 μl, Fraction 3; 20 μl, 2×10^{-3} M dT (0·45 μc/ml); 50 μl, 5×10^{-2} M ATP; and 20 μl, 7×10^{-3} M (metal salt or EDTA); total volume 140 μl. All reagents were in 0·2 M phosphate buffer (pH 7·0). Reaction mixtures were incubated for 15 min at 30°.

A number of pyrimidine nucleosides and deoxynucleosides were tested for their ability to be phosphorylated by peanut thymidine kinase preparations (Table 4). The assays were carried out in the usual manner with the substitution of the various other substrates for dT and by using partially purified enzyme (Fraction 3, Table 2); reaction products were identified in each case by chromatographic procedures. All of the nucleosides tested were phosphorylated by the kinase preparation; however, phosphorylation of the deoxyribonucleosides was greater than that of the ribonucleosides by a factor of approximately two to one. The probable presence of pyrimidine nucleoside and deoxynucleoside phosphatases makes it impossible to speculate as to the efficiency of phosphorylation of the various nucleosides by the enzyme or enzymes present. Wanka and Bauer[23] reported that in corn, deoxycytidine (dC) was phosphorylated nearly as fast as dT but the rate of cytidine (C) phosphorylation was about one-fourth the dC rate. They also found that uridine (U) and deoxyuridine (dU) could be

[26] J. E. CLEAVER, *Thymidine Metabolism and Cell Kinetics*, p. 50, John Wiley, New York (1967).

[27] T. DUAFALA, *The Role of Thymidine Kinase in DNA Synthesis*, p. 16, UCRL-50571, Clearinghouse for Federal Scientific and Technical Information, National Bureau of Standards, U.S. Department of Commerce, Springfield, Virginia (1969).

phosphorylated by their corn extracts. Deoxyuridine, dT and the 5-halogenated deoxyuridines acted as substrates for thymidine kinase from Walker tumor,[7] but this enzyme was unable to phosphorylate U, C and dC similar to the substrate specificity reported for thymidine kinase from *E. coli*.[20]

If the substrates (Table 4) were added as unlabeled components to reaction mixtures phosphorylating ^{14}C-dT, TMP formation was inhibited with increasing concentrations of the above (Table 5). Thymidine kinase activity found in plants[16] and in animal tumors[7] is

TABLE 4. PHOSPHORYLATION OF PYRIMIDINE NUCLEOSIDES BY A PEANUT THYMIDINE KINASE PREPARATION

Radioactive substrate	Activity	% of dT activity
dT	1·24	100
dC	0·87	70·2
C	0·47	37·9
dU	1·06	85·5
U	0·59	47·6

The reaction mixtures consisted of 50 μl, Fraction 3; 20 μl, 2×10^{-3} M substrate (0·45 μc/ml); 50 μl, ATP 5×10^{-2}, MgCl 4×10^{-3} M; all reagents in 0·2 M phosphate buffer (pH 7·0); total volume 120 μl. Reaction mixtures were incubated for 15 min at 30°.

TABLE 5. INHIBITION OF THYMIDINE PHOSPHORYLATION BY VARIOUS PYRIMIDINE NUCLEOSIDES

	Per cent inhibition Nucleoside concentration (M)			
Nucleoside	$1{\cdot}4 \times 10^{-2}$	$1{\cdot}4 \times 10^{-3}$	$2{\cdot}9 \times 10^{-4}$	$2{\cdot}9 \times 10^{-5}$
dC	78·1	25·3	10·1	2·2
C	60·2	13·4	8·1	0
dU	85·4	24·2	6·8	−5
U	62·8	14·9	4·2	2·5

The assay components consisted of 50 μl, Fraction 3; 20 μl, 2×10^{-3} M dT (0·45 μc/ml); 50 μl, 5×10^{-2} M ATP, 4×10^{-3} M MgCl; 20 μl (various non-isotopic nucleosides); all reagents in 0·2 M phosphate buffer (pH 7·0); total volume 140 μl. Reaction mixtures were incubated for 20 min at 30°.

inhibited by pyrimidine ribo- and deoxyribonucleosides, respectively. Deoxyuridine was shown to competitively inhibit dT phosphorylation in Walker tumor.[7] Wanka[16] demonstrated a mutual competitive inhibition of dT and U phosphorylation by U and dT respectively. His assumption was that this condition should exist if a single enzyme was involved in the phosphorylation of both substrates.

The data presented here cannot distinguish with certainty between the presence of a single non-specific pyrimidine nucleoside–deoxynucleoside kinase capable of phosphorylating all of the substrates tested or the presence of multiple specific kinases. Ratios of dT to U phosphorylation remained constant throughout our purification procedures, suggesting that a single enzyme might be involved. Obviously, however, multiple kinases could have

been simultaneously purified. After extensive physiological and biochemical characterizations of thymidine kinase in corn, Wanka and Bauer[23] have suggested that the enzyme that is responsible for dT phosphorylation is nonspecific to the extent that it can also phosphorylate U, C, dU and dC.

DISCUSSION

The thymidine kinase activity found in germinating peanuts has been partially characterized. An attempt to purify the enzyme was only partially successful and the competing reactions found in crude preparations were not eliminated. Phosphatase activity capable of dephosphorylating TMP to dT and removing all phosphate from ATP was present in all preparations. The relatively high concentrations of phosphate used in other plant systems[1,2] failed to inhibit the dephosphorylations. Thus, the kinase activities presented comprise net rather than absolute rates. Thymidine kinase from peanuts proved to be relatively heat stable, a characteristic it shares with thymidine kinase from other sources.[2,20] Partially purified enzyme extracts were shown to be able to phosphorylate U, dU, C and dC, a property also common to the thymidine kinase from corn.[16,23] However, unlike the thymidine kinase from corn, we were unable to separate peanut thymidine kinase into two subunits using comparable techniques. Wanka and Bauer[23] have suggested that a single non-specific nucleoside–deoxynucleoside kinase was responsible for the phosphorylation of all of the above-mentioned substrates in corn. Although the phosphorylation of dT was inhibited by U, dU, C and dC, the data does not distinguish between the presence of single or multiple phosphorylating enzymes. The phosphorylating activity of thymidine kinase from mammalian tumors[7] and bacteria[20] is confined to dT, dU and dC analogues. Such specificity differences might indicate different physiological roles for the plant and animal enzymes.

EXPERIMENTAL

Enzyme Source

Peanut seeds (*Arachis hypogaea* var. N.C.2) were dusted with tetramethylthiuramdisulfide (75 per cent powder) and placed between layers of moist paper towels which were subsequently rolled to form scrolls. The scrolls were placed in a vertical position in a germinator and the seeds were allowed to develop for 120–168 hr at 25° in the dark with occasional watering.

Preparation of Crude Enzyme Extracts

At harvest, seedlings were thoroughly rinsed in distilled water and treated for 10 min in 1·5% NaOCl and again rinsed. Crude homogenates were prepared in cold (0–4°) potassium phosphate buffer, 0·2 M, pH 7·0, by grinding in a Servall Omni-mixer for 1 min at full speed. The resulting brei was then passed through several layers of fine-mesh nylon cloth and centrifuged in the cold at 32,000 *g* for 30 min. The resulting supernatant was used as the crude enzyme source.

Preparation of Acetone Powders

Hypocotyls were excised from the seedlings and surface-sterilized with NaOCl (1·5 per cent) for 10 min. They were then homogenized at full speed in −10° acetone for 90 sec. The resulting slurry was washed on a Buchner funnel with additional acetone until the effluent was clear. The white compact mat remaining on the filter paper was then washed with ether (0–4°) to remove the acetone, and then dried at room temp.

Protein Determination

One milliliter samples were precipitated with an equal volume of 10 per cent trichloroacetic acid (TCA) and placed in an ice bath for 30 min. They were then centrifuged before washing the precipitates twice with 10 per cent TCA. The final pellets were dissolved in 0·1 M NaOH. Duplicate samples were assayed for protein according to the method of Lowry *et al.*[28] Standard curves were prepared with BSA.

[28] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).

Assay Procedure

The method used was a modification of procedures by Hotta and Stern,[1] Wanka and Walboomers[24] and Lucas.[29] The assay mixture (total volume, 120 μl) consisted of: ^{14}C-dT (2×10^{-3} M) (0·45 μc/ml) 20 μl; ATP (5×10^{-2} M) and $MgCl_2$ (4×10^{-3} M) 50 μl; enzyme preparation 50 μl; all in sodium–potassium phosphate buffer (pH 7·0) giving a final concentration of 0·2 M. The protein content did not exceed 2 mg/ml. Other labeled pyrimidine nucleoside and deoxynucleoside substrates were substituted for dT at identical concentrations and specific activities. The final volume was increased by 20μl in those cases requiring the addition of other components. The reaction mixtures were incubated for various times at 30° unless indicated otherwise. The reactions were terminated by the addition of 300 μl of methanol and rapid cooling to 4° in an ice bath. The assay mixtures were then centrifuged to sediment the precipitates. The supernatants were spotted on Whatman No. 3 MM paper and chromatographed in a water-saturated butanol solvent. Developed chromatograms were air-dried before detecting the nucleosides and nucleotides with short-wave u.v. light. These fractions were cut from the chromatograms and counted in a scintillation counter. Enzyme activity was determined as the per cent nucleoside phosphorylated per minute. Specific activity was calculated as per cent nucleoside phosphorylated per minute per milligram protein. All activity values are the average of four to eight replications unless otherwise indicated. Portions of buffers, substrates, cofactor solutions and enzyme preparations were frequently monitored for bacterial contamination.

PEI-Cellulose Chromatography

Qualitative studies of the reaction products were conducted on thin-layer polyethyleneimine cellulose chromatograms with H_2O and LiCl 1 M as solvents according to the methods of Randerath and Randerath[21] and Lucas.[29]

[29] Z. J. LUCAS, *Science* **156**, 1237 (1967).