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PURIFICATION AND PROPERTIES OF POLYNUCLEOTIDE KINASE OF CALF THYMUS

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Summary

Polynucleotide kinase (ATP:5′-dephosphopolynucleotide 5′-phosphotransferase, EC 2.7.1.78) has been purified approx. 1500-fold from calf thymus. This enzyme phosphorylates 5′-hydroxyl termini in DNA using ATP as phosphate donor. RNA is phosphorylated at a much lower rate than DNA. The reaction requires the presence of a divalent cation, preferably Mg^{2+} or Mn^{2+} and is sensitive to sulfhydryl antagonists. The optimum pH for enzyme activity is 5.5. Enzyme activity is inhibited by low concentrations of inorganic sulfate and by some sulfate polymers. The kinase-catalyzed incorporation of the terminal phosphate of ATP into polynucleotides is inhibited by other nucleoside and deoxynucleoside triphosphates. The enzyme molecule has a molecular weight of about 70 000 and a Stokes radius of 4.3 nm. It has a frictional ratio of 1.44 indicating an asymmetrical structure. Calf thymus tissue should provide a useful alternative source for preparation of mammalian polynucleotide kinase.

Introduction

Polynucleotide kinase (ATP:5′-dephosphopolynucleotide 5′-phosphotransferase, EC 2.7.1.78), an enzyme which catalyzes the transfer of the terminal phosphate from a nucleoside 5′-triphosphate to the 5-hydroxyl terminus of polynucleotides was first isolated from extracts of *Escherichia coli* cells infected by bacteriophages T2 or T4 [1,2]. Novogrodsky et al. [3] and later Ichimura and Tsukada [4] detected an apparently similar activity in crude extracts of rat liver, but Levin and Zimmerman [5] recently showed that the liver enzyme is active solely on DNA and higher molecular weight oligonucleotides. In the course of studies dealing with DNA repair processes we have obtained and extensively purified a polynucleotide kinase from calf thumus. The calf thymus enzyme catalyzes the phosphorylation of 5′-hydroxyl termini in single- or double-stranded DNA and is much less active on RNA. The purification and some of the properties of calf thymus polynucleotide kinase are described in this communication.

Experimental procedure

Materials. T4 polynucleotide kinase and micrococcal nuclease were obtained from P-L Biochemicals. Crystallized pancreatic DNAase, and calf thymus DNA were obtained from Worthington Biochemical Corp. [γ-^{32}P]ATP (10—25 Ci/nmol) was purchased from ICN. Pronase, heparin and iodoacetic acid were purchased from Calbiochem. Hydroxyapatite and chemicals for acrylamide gel electrophoresis were purchased from Bio-Rad Laboratories. Sulfopropyl-Sephadex was obtained from Pharmacia Fine Chemicals. Protamine sulfate was obtained from Eli Lilly. Wheat germ RNA was purchased from P-L Biochemicals. ^{32}P-labeled nicked calf thymus DNA was prepared by the method of Richardson [1].

Methods. Polynucleotide kinase was routinely assayed by determining the incorporation of the gamma phosphate from [γ-^{32}P]ATP into DNA to form acid-precipitable counts. The reaction mixture (0.3 ml) contained 50 μg calf thymus DNA containing nicks with 5'-OH termini, 13 μM [γ-^{32}P]ATP (20 counts · min^{-1} · pmol^{-1}), 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 50 mM sodium acetate (pH 5.5), 30 μg bovine serum albumin and enzyme. After incubation for 20 min at 37°C the samples were placed on ice. Bovine serum albumin (350 μg) in 0.7 ml of 0.014 M sodium pyrophosphate was added and the DNA was precipitated by 1 ml of 20% trichloroacetic acid. The pellets obtained by centrifugation were washed twice by dissolution in 1 ml of 0.1 M NaOH and reprecipitated with 2 ml of 10% trichloroacetic acid. The final precipitates were dissolved in 0.3 ml of 1 M NaOH and added to scintillation vials which held PCS counting cocktail (Amersham). The assay tubes were rinsed with 0.2 ml water which was added to the vials followed by 0.5 ml of 1 M HCl. Samples were counted in a Packard liquid scintillation spectrometer. One unit of kinase activity was defined as that amount converting 1 nmol of ^{32}P into acid-precipitable material.

Nicked DNA containing 5'-hydroxyl termini for use in the kinase assay was routinely prepared by partially digesting native calf thymus DNA with micrococcal nuclease as described by Richardson [1]. In a typical preparation calf thymus DNA (8 mg) was treated at 37°C with 75 units of micrococcal nuclease for sufficient time to render the DNA 30% acid soluble. The reaction was then terminated by chilling the mixture to 0°C and the DNA was dialyzed for 12 h against 1 M KCl and then for 24 h against 0.02 M KCl. A typical preparation made in this manner was heterogeneous in size with molecules ranging from oligonucleotides to 150 base pairs as shown by agarose gel electrophoresis performed by the method of Sharp et al. [6].

Salt concentrations of column fraction were determined by conductivity measurements. Protein concentrations were determined by the method of Lowry et al. [7] after precipitation of the samples with cold 10% trichloroacetic acid. Bovine serum albumin was used as the standard in protein determinations.

Determination of sedimentation coefficient of the enzyme. The sedimentation coefficient of kinase was determined by rate zonal density gradient centrifugation in 5—20% sucrose gradients at 4°C as described by Martin and Ames [8]. Gradients contained 0.01 M potassium phosphate, pH 7.2, 0.2 M KCl, 1 mM dithiothreitol, and 0.1 mM EDTA. Enzyme (2 units) or a standard protein

in 0.2 ml buffer without sucrose was layered onto a 4.8 ml linear gradient and centrifuged for 20 h at 48 000 rev./min in a Beckman SW 65 rotor. Standards employed were ovalbumin ($s_{20,w}$ = 3.55 S) [9], bovine serum albumin ($s_{20,w}$ = 4.31 S) [10] and aldolase ($S_{20,w}$ = 7.82 S) [11]. (The ovalbumin and aldolase were from a Calibration Kit for molecular weight determination purchased from Pharmacia Fine Chemicals). Location of standards in the gradients was determined by absorbance at 280 nm.

Determination of Stokes radius of the enzyme. This parameter was estimated by gel filtration as described by Ackers [12]. Chromatography was carried out at 4°C using a 0.64 cm^2 × 60 cm column of Sephacryl S-200 Superfine (Pharmacia Fine Chemicals) in 0.01 M Tris · HCl (pH 7.2) which contained 1 mM dithiothreitol, 0.1 mM EDTA and 25% glycerol. The column was calibrated using blue dextran and [γ-^{32}P]ATP. Standards employed and their reported Stokes radii [13] were: RNAase A (1.75 nm), chymotrypsinogen A (2.2 nm), ovalbumin (3.0 nm), bovine serum albumin (3.5 nm), and aldolase (4.6 nm). Kinase (0.2 unit) and standards were applied in a volume of 0.4 ml. Fractions (0.5 ml) were collected and assayed for kinase activity. Standards were determined by absorbance at 280 nm. Results were confirmed using Sephadex G-200 (Pharmacia) under the same conditions.

Results

Purification of polynucleotide kinase from calf thymus

The major purification steps and results of a typical purification are shown in Table I. All operations were carried out at 0—4°C. The activity in the crude extracts is not all DNA dependent and probably contains other kinases in addition to polynucleotide kinase. However, the activity in fractions III through VI is entirely dependent on added nicked DNA.

Crude extracts and protamine sulfate fractionation. A crude extract was obtained by a procedure previously employed to obtain RNA polymerase and DNA polymerase from calf thymus [14]. Calf thymus (400 g) was minced in two volumes of 0.05 M Tris · HCl (pH 7.7), 0.005 M $MgCl_2$, 0.005 M 2-mercaptoethanol and homogenized in a Waring blender at 0°C for 60 s at medium speed

TABLE I

PURIFICATION OF POLYNUCLEOTIDE KINASE FROM CALF THYMUS

Values are results of a typical purification. Description of assay conditions and definition of units are found in the text.

Fraction and step	Protein (mg)	Activity (units)	Specific activity (units/mg protein)	Yield (%)
I. Crude	25 700	1605	0.055	100
II. Protamine sulfate	4 200	670	0.16	42
III. Phosphocellulose	270	240	0.76	13
IV. Hydroxyapatite	91	184	2.9	11
V. Sulfopropyl-Sephadex	5.1	98	19.2	6.1
VI. Glycerol gradient	0.7	60	86	3.7

and 60 s at high speed. This material was then centrifuged for 10 min at 10 000 × *g* in a Beckman J21B centrifuge. The supernatant was filtered through two layers of cheesecloth and centrifuged at 78 000 × *g* for 1 h in the 30 rotor of a Beckman L4 ultracentrifuge. The supernatant (Fraction I) was again filtered through cheesecloth to remove fat and mixed for 15 min with 0.3 volumes of 0.5% protamine sulfate. The mixture was centrifuged for 15 min at 10 000 × *g* and the supernatant, which contained the bulk of the activity, was subjected to ammonium sulfate fractionation. Material which precipitated in the 25—55% $(NH_4)_2SO_4$ fraction was resuspended in a minimum volume of 0.02 M potassium phosphate (pH 7.2), 0.003 M 2-mercaptoethanol and dialyzed overnight against the same buffer (Fraction II).

Phosphocellulose chromagraphy. Dialyzed Fraction II was applied to a phosphocellulose column, 10 cm^2 × 20 cm, equilibrated with the same buffer, at a protein concentration of 5 mg per ml of phosphocellulose. The enzyme was eluted using a linear 0.01—0.6 M KCl gradient. Peak fractions, which were eluted at about 0.2 M KCl, were pooled (Fraction III) and concentrated by precipitation with 60% (w/v) solid $(NH_4)_2SO_4$ and dialyzed against 0.02 M potassium phosphate, pH 7.0, 0.003 M mercaptoethanol.

Hydroxyapatite chromatography. Dialyzed Fraction III enzyme was then applied to a 5 cm^2 × 12 cm column which contained a 1 : 1 mixture of hydroxyapatite and Whatman CF 11 cellulose powder and which had been equilibrated with the same buffer. Elution was performed using a 250 ml linear potassium phosphate gradient (0.05—0.5 M). Fractions containing enzyme activity which emerged at about a 0.22 M potassium phosphate were combined and concentrated by precipitation with 60% solid $(NH_4)_2SO_4$ (Fraction IV).

Sulfopropyl-Sephadex chromatography. Sulfopropyl-Sephadex C-50 was equilibrated with 0.01 M potassium phosphate containing 0.003 M 2-mercaptoethanol, and 2.5 cm^2 × 12 column was poured and washed with the same buffer. Fraction IV was dialyzed against this buffer and then applied to the column. Elution was performed using a 150 ml linear KCl gradient (0.01—0.5 M). The kinase activity emerged in a sharp peak at 0.18 M KCl. The active fractions were combined, precipitated with $(NH_4)_2SO_4$ and the pellet resuspended as a slurry in a minimal volume of potassium phosphate buffer, pH 7.0 (Fraction V). In some cases the active fractions from the column were frozen directly without $(NH_4)_2SO_4$ precipitation and stored at —80°C.

Glycerol gradient centrifugation. Enzyme (Fraction V) was dialyzed for 4 h against glycerol gradient buffer, which consisted of 0.01 M potassium phosphate (pH 7.5), 0.2 M KCl, 0.005 M dithiothreitol and 0.0001 M EDTA. Glycerol gradients (10—30%, 5 ml) were prepared in tubes of an SW 50L rotor and the dialyzed enzyme was layered onto the gradients. The samples were centrifuged for 16 h at 188 000 × *g*. Fractions were collected dropwise from the bottom of the tube and analyzed for polynucleotide kinase activity and protein concentration. Tubes containing activity were pooled and frozen at —80°C (FractionVI).

Stability of the various fractions. Crude extracts were not routinely stored for more than 1—2 days. The protamine sulfate supernatant (Fraction II) was relatively stable at 0°C as an $(NH_4)_2SO_4$ slurry, losing about 25—50% of its activity in 1 month. Fraction III from the phosphocellulose column was gener-

TABLE II

REQUIREMENTS FOR ENZYME ACTIVITY

The complete reaction mixture contained the standard assay ingredients with 8 μg of enzyme (Fraction IV). Kinase activity was determined as described in Methods except for the changes noted.

Components	Kinase activity (pmol/20 min)
Complete	26.9
Omit incubation at 37°C	<0.5
—enzyme	<0.5
—$MgCl_2$	2.0
—$MgCl_2$ (add 10 mM $MnCl_2$)	35.4
—$MgCl_2$ (add 0.3 mM $ZnCl_2$)	8.9
—nicked DNA	<0.5
—nicked DNA, add untreated DNA	1.1
—bovine serum albumin	27.5
—mercaptoethanol	22.8
Enzyme dialyzed vs. 0.05 M Tris · HCl/20% glycerol, pH 7.2	24.1
Dialyzed enzyme, omit mercaptoethanol	3.2

ally, but not always, stable at 0°C as an $(NH_4)_2SO_4$ slurry, showing little or no loss of activity for periods of up to 3 months. Occasional preparations lost activity precipitously when stored in this way. An $(NH_4)_2SO_4$ slurry of the hydroxyapatite column-purified enzyme (Fraction IV) showed the same stability as Fraction III. Fraction V could be stored for short periods as an $(NH_4)_2SO_4$ slurry with variable loss of activity but was most stable at —80°C where it lost 20—30% of its activity per week. Fraction VI was also most stable when stored at —80°C losing 20—40% of its activity per week under these conditions.

Other enzyme activities in the preparation. Some preparations of Fraction III contained low levels of polynucleotide ligase activity when assayed by the method of Weiss et al. [15], using ^{32}P-labeled, nicked DNA prepared (see Methods) using calf thymus kinase (Fraction V) or using rat liver DNA kinase prepared as described by Ichimura and Tsukada [4]. However, assays of 0.1 unit of calf thymus kinase Fractions IV through VI for ligase activity revealed less than $2 \cdot 10^{-3}$ pmoles of ^{32}P internalized in the DNA per 20 min at 37°C.

Incubation of 0.1 unit of kinase (Fractions IV—VI) with 20 μg of ligase substrate in the absence of [γ-^{32}P]ATP for 1 h at 37°C revealed less than 1% solubilization of ^{32}P counts, indicating the absence of significant nuclease or phosphatase activities which might interfere with the measurement of kinase activity.

Minimal requirements for enzyme activity. Table II illustrates the requirements for kinase activity. The enzyme requires the presence of a suitable phosphate acceptor, in this case DNA containing nicks with 5′-terminal hydroxyl groups (Fractions III—VI are completely dependent upon the addition of exogenous template). A divalent cation is required for kinase activity but this requirement can be satisfied by a number of metals of which Mg^{2+} and Mn^{2+} support maximal rates of activity. Whereas 10 mM is the optimal concentration of Mg^{2+} or Mn^{2+}, the optimal cation concentration using Zn^{2+} is 0.3 mM. The addition of bovine plasma albumin is not required for activity but improves the reproducibility of the assay, as was reported by Levin and Zimmerman [5] for rat liver kinase. The requirements for mercaptoethanol become evident if the

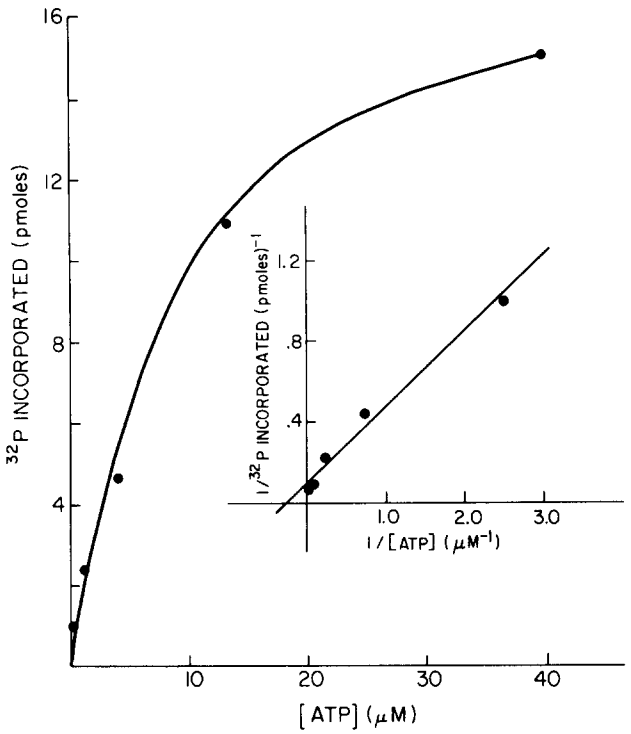


Fig. 1. Effect of ATP concentration on kinase activity. Assays employing Fraction IV enzyme were carried out as described in Methods, except that a range of ATP concentrations was used as indicated. Specific activity of ATP = 26 cpm/pmol. K_m for ATP calculated from the double reciprocal plot = 3.9 μM.

enzyme is dialyzed to remove traces of this substance in the enzyme preparation before the assays are done.

When the assays are carried out at 37°C under standard conditions the reaction rate is constant for 20 min after which it decreases. Little or no further phosphorylation occurs after 1 h.

Phosphate donor and acceptor requirements. ATP was the only phosphate donor tested in these studies. Under standard reaction conditions the K_m for ATP is 3.9 μM (Fig. 1).

DNA is the preferred phosphate acceptor for the calf thymus kinase. The reaction rate is proportional to DNA concentration but the absolute amount of DNA required varies according to the number of nicks in the particular DNA preparation being used as substrate. The relative abilities of single- and double-stranded DNA and RNA to serve as substrates for the calf thymus kinase are shown in Table III. In contrast to the bacteriophage enzyme for which nicked RNA containing 5′-hydroxyl termini is as good a phosphate acceptor as nicked DNA, the calf thymus enzyme phosphorylates nicked DNA more than 10 times as efficiently as nicked RNA. Both single-stranded and double-stranded nicked DNAs are equally good substrates.

Effects of pH and salt concentration on enzyme activity. The variation in calf thymus kinase activity as a function of pH is shown in Fig. 2. Enzyme ac-

TABLE III

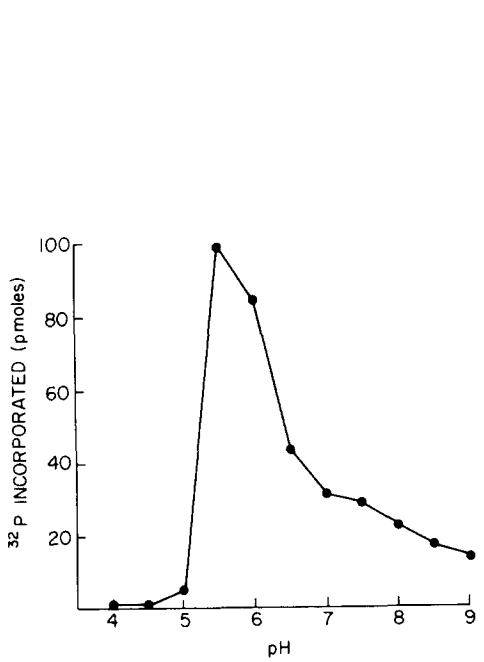
ATIVITY OF KINASE ON DNA AND RNA

Calf thymus kinase was assayed as described in Methods except for the use of different substrates as noted. Bacteriophage T4 kinase was assayed as described by Richardson [1].

Substrate	Kinase activity (pmol)	
	Calf thymus enzyme	T4 enzyme
DNA, untreated, native	2	3.5
DNA, micrococcal nuclease-treated, native	113	74
DNA, micrococcal nuclease-treated, denatured	121	71
RNA, micrococcal nuclease-treated, native	11	104
RNA, micrococcal nuclease-treated, denatured	10	99

tivity shows a fairly sharp optimum at about 5.5 but some activity remains at pH values as high as 9.0. Kinase activity is maximum at low ionic strength. Concentrations of monovalent salts above 0.02 M inhibit kinase activity as shown in Fig. 3. Similar profiles are observed using either NaCl or KCl.

Nature of the phosphorylated product. The ^{32}P counts incorporated into DNA by calf thymus kinase are rendered more than 99% acid soluble by treat-



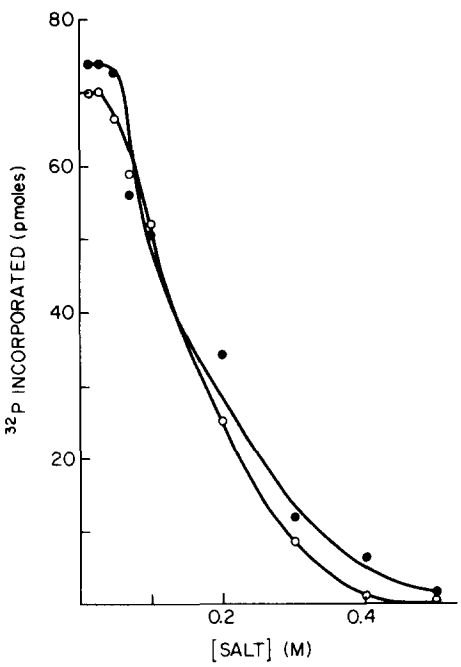


Fig. 2. Kinase activity as a function of pH. Aliquots of enzyme (Fraction IV) were assayed under normal conditions except that the pH was varied as indicated. Sodium acetate buffer was used from pH 4.0 to 6.5, Tris/glycine from pH 6.5 to 9.0.

Fig. 3. Effect of monovalent salt concentration on kinase activity. Assays were carried out in the presence of 0.01 M sodium acetate buffer (pH 5.0). Enzyme (Fraction IV) was dialyzed against the same buffer to remove salts before being assayed. ●———●, KCl; ○———○, NaCl.

TABLE IV

SITE ON DNA PHOSPHORYLATED BY KINASE

^{32}P-labeled DNA was prepared by calf thymus kinase as described in Methods. Aliquots of labeled DNA containing 500 counts of ^{32}P were incubated with the enzymes indicated for 30 min at 37°C in 0.2 ml of 0.05 M Tris · HCl (pH 8.0) which contained (except in the case of alkaline phosphatase) 0.01 M $MgCl_2$. Determination of ^{32}P counts released in Norit non-adsorbable form was performed as described by Richardson and Kornberg [16].

Enzyme treatment	^{32}P rendered non-adsorbable to Norit (%)
Buffer alone	3
Alkaline phosphatase (50 μg)	91
Snake venom phosphodiesterase (100 μg)	2
5′-Nucleotidase (100 μg)	7
Venom phosphodiesterase (100 μg) + 5′-nucleotidase (100 μg)	94

ment with pancreatic DNAase (50 μg/ml for 30 min at 37°C), micrococcal nuclease (300 units/ml for 30 min at 37°C), or *E. coli* alkaline phosphatase (50 μg/ml for 30 min at 37°C). Conversely the radioactivity incorporated into DNA by kinase is not rendered acid soluble (less than 10%) by treatment with pronase (50 μg/ml for 30 min at 37°C) pancreatic ribonuclease (20 μg/ml for 30 min at 37°C) of 1 M NaOH for 30 min at 37°C. When nicked RNA is used as substrate, the ^{32}P radioactivity in the product is solubilized by ribonuclease or NaOH but not by DNAase or pronase.

Table IV illustrates the results of data which prove that the calf thymus enzyme phosphorylates 5′-hydroxyl termini in the DNA. In this experiment nicked DNA phosphorylated by calf thymus polynucleotide kinase was subjected to treatment with several enzymes and the percent of counts rendered acid soluble and Norit non-adsorbable was determined. Alkaline phosphatase liberated almost all of the ^{32}P counts as Norit non-adsorbable phosphate showing that the ^{32}P was located at 3′- or 5′-termini of the DNA. Neither snake venom phosphodiester nor 5′-nucleotidase alone converted the ^{32}P in the kinase product to an acid-insoluble, Norit non-adsorbable form. However, the combined action of these two enzymes converted more than 90% of the ^{32}P radioactivity to inorganic phosphate. These results demonstrate that the kinase transfers the γ-phosphate of ATP to 5′-hydroxyl termini on the acceptor DNA.

Inhibition of kinase activity by sulfates and other compounds. Table V illustrates the effects on calf thymus polynucleotide kinase activity of a number of compounds which inhibit kinases obtained from other sources. Inorganic sulfate strongly inhibits the calf thymus enzyme although the inhibition is less striking than that reported for rat liver kinase [5,17]. Similar degrees of inhibition are obtained using $(NH_4)_2SO_4$ and Na_2SO_4 confirming that the inhibition is due to sulfate and not to the cation employed. Some sulfur-containing polymers such as heparin inhibit calf thymus kinase whereas others such as agar do not (a difference from the bacteriophage enzyme which is inhibited by both heparin and agar). Sulfhydryl inhibitors such as iodoacetate and $AgNO_3$ also inhibit the enzyme; the inhibition is prevented by the addition of 2-mercaptoethanol to the reaction mixture. Sodium pyrophosphate is a fairly potent in-

TABLE V

SENSITIVITY OF KINASE TO VARIOUS INHIBITORS

Assays were performed as described in Methods. Control incorporation was 60 pmol per 10 min for undialyzed enzyme and 19 pmol per 10 min for dialized enzyme. Dialysis of enzymes was for 2 h at 4°C against 0.05 M Tris · HCl (pH 7.2), 0.003 M $MgCl_2$, 20% glycerol. The amount of Fraction V enzyme used was 0.06 unit per assay.

Inhibitor	Concentration	Kinase activity (percent of control)
$(NH_4)_2SO_4$	10 mM	40
$(NH_4)_2SO_4$	30 mM	20
Heparin	1 mg/ml	25
Agar	3 mg/ml	109
Sodium pyrophosphate	20 mM	3
Iodoacetate [a]	0.3 mM	<1
$AgNO_3$ [a]	0.3 mM	<1
CTP, GTP or UTP	0.3 mM	<10
dATP, dGTP or dTTP	0.3 mM	<5
dCTP	0.3 mM	20

[a] Enzyme dialyzed and assayed in the absence of 2-mercaptoethanol.

hibitor of enzyme activity. Low concentrations of ribonucleoside and deoxyribonucleoside triphosphates inhibit kinase activity, probably by competing with the labeled ATP. This suggests that these compounds may be able to serve as alternate substrates for polynucleotide kinase, but this possibility has not been investigated directly. Deoxycytidine triphosphate is a slightly weaker inhibitor on a molar basis than are the other deoxynucleoside triphosphates.

Physical properties of calf thymus kinase. The sedimentation coefficient of calf thymus was determined by rate zonal centrifugation in 5—20% sucrose gradients containing 0.2 M KCl. By comparison with standards of known sedimentation coefficient calf thymus kinase was found to have an $s_{20,w}$ of 4.3 S under the conditions employed. The Stokes radius of the enzyme was determined by gel filtration chromatography in buffer containing 0.2 M KCl essentially as described by Ackers [12]. By comparison with standards of known Stokes radii, a value of 3.9 nm was obtained for the Stokes radius of calf thymus kinase. Using these experimentally determined values for sedimentation coefficient and Stokes radius and assuming [11] a partial specific volume for the enzyme of 0.725 cm^3/g, the molecular weight and frictional coefficient of kinase were calculated as described by Siegel and Monty [18]. The molecular weight was determined to be 70 000, somewhat greater than that which would be expected for a spherical molecule with the same sedimentation coefficient. The frictional ratio (f/f_0) was calculated to be 1.44 indicating that kinase is an elongated molecule; (a prolate or oblate elipsoid with this frictional ratio, would have an axial ratio greater than ten [19]).

Analysis of the size of subunits of the enzyme was performed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using the method of Laemmli [20]. Fraction V enzyme was applied to a glycerol gradient and aliquots of the fractions were assayed for enzyme activity or subjected to gel electrophoresis. A prominent band of molecular weight approx. 70 000—75 000 was seen in the gradient fractions with highest enzyme activity. Analysis of Fraction V enzyme in 7.5% polyacrylamide gels which were run at a pH close

to neutrality according to the method of Davis [21], indicates a prominent major band and several accompanying minor bands. The results of the gel electrophoresis studies suggest that while the kinase preparations are highly purified, they are not homogeneous and that the enzyme itself is probably monomeric.

Discussion

This communication confirms and extends the available information about mammalian polynucleotide kinases. Until now the only mammalian enzyme of this type to have been studied was the rat liver DNA kinase. The finding of an enzyme in calf thymus with generally similar properties to those of the rat liver enzyme suggest that polynucleotide kinase may be of univeral occurrence in mammals and perhaps in all eukaryotes. This possibility is further supported by the reported detection [22] of a polynucleotide kinase activity in crude extracts of human lymphocytes.

Although the levels of kinase detectable in crude extracts of calf thymus are lower than those reported in extracts of rat liver nuclei, the purification procedure described here is suitable for use with large amounts of tissue and yields enzyme of comparable specific activity to that so far obtained from rat liver. The ready availability of calf thymus makes it a convenient alternative source for mammalian kinase. Calf thymus enzyme appears to have less complete specificity for DNA (over RNA) than the rat liver enzyme which may make it of some use in phosphorylating RNA. In addition, since calf thymus is an often-used source for other enzymes involved in DNA metabolsm it will be useful to have available a polynucleotide kinase from the homologous tissue.

As expected, calf thymus kinase resembles more closely in its properties the enzyme from rat liver than the kinase found in bacteriophage-infected *E. coli*. For example, the pH optimum of calf thymus and rat liver enzymes is 5.5 as opposed to the pH optimum of 7.4—8.0 shown by bacteriophage enzyme. Likewise the effects of monovalent cations and inorganic and organic sulfates on enzyme activity are similar, although not identical, for both mammalian enzymes. As mentioned above an exception to the similarity of the calf thymus and rat liver enzymes appears to be the ability of the thymus enzyme to phosphorylate RNA, albeit at low rates relative to DNA. (The presence of low levels of a separate RNA kinase in the calf thymus preparation is unlikely but has not been completely ruled out).

Although it seems likely that phosphorylation of 5′-hydroxyl termini of DNA and RNA may be important in processes such as DNA synthesis and repair and RNA processing, the physiological role of polynucleotide kinase in vivo remains unknown. In prokaryotes the enzyme has so far only been discovered in bacteriophage T2- and T4-infected *E. coli*. Kinase-deficient mutants of T4 which have been examined to date have been capable of normal DNA replication, recombination and repair [23]. In eukaryotes even less is known. Our observations that physiologic levels of UTP inhibit kinase activity suggests that nucleoside triphosphate levels may modulate activity in some tissues and perhaps inhibit the enzyme under certain conditions. Aside from an observation by Ichimura and Tsukada [4] that levels of liver kinase were unchanged from control levels at one point during the course of liver regeneration, nothing is

known concerning possible changes in liver kinase levels during periods of DNA replication or repair. A report that kinase activity in crude extracts of human lymphocytes was increased 2-fold following phytohemagglutinin treatment [24] raises the possibility that the lymphocyte enzyme may be involved in DNA replication. Our own observations (Austin, G.E. and Moyer, G.H., unpublished), suggest that kinase activity may be higher in the thymus tissue of very young calves than in older animals, perhaps reflecting the more active DNA synthesis that is known to take place in this organ during early developement.

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References

1 Richardson, C.C. (1965) Proc. Natl. Acad. Sci. U.S. 54, 158—165
2 Novogrodsky, A. and Hurwitz, J.J. (1966) J. Biol. Chem. 241, 2923—2932
3 Novogrodsky, A., Tal, M., Traub, A. and Hurwitz, J. (1966) J. Biol. Chem. 241, 2933—2943
4 Ichimura, M. and Tsukada, K. (1971) J. Biochem. 69, 823—828
5 Levin, C.J. and Zimmerman, S.B. (1976) J. Biol. Chem. 251, 1767—1774
6 Sharp, P.A., Sugden, B. and Sambrook, J. (1973) Biochemistry 12, 3055—3063
7 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
8 Martin, R.G. and Ames, B.N. (1961) J. Biol. Chem. 236, 1372—1379
9 Lamm, O. and Polson, A. (1936) Biochem. J. 30, 528—541
10 Shulman, S. (1953) Arch. Biochem. Biophys. 44, 230—240
11 Kawahara, K. and Tanford, C. (1966) Biochemistry 5, 1578—1584
12 Ackers, G.K. (1964) Biochemistry 3, 723—730
13 Tanford, C., Nozaki, Y., Reynolds, J.A. and Makino, S. (1974) Biochemistry 13, 2369—2376
14 Furth, J.J., Nicholson, A. and Austin, G.E. (1970) Biochim. Biophys. Acta 213, 124—133
15 Weiss, B., Live, T.R. and Richardson, C.C. (1968) J. Biol. Chem. 243, 4530—4542
16 Richardson, C.C. and Kornberg, A. (1964) J. Biol. Chem. 239, 242—250
17 Teraoka, H., Mitua, K., Sato, F., Shimoyachi, M. and Tsukada, K. (1975) Eur. J. Biochem. 58, 297—302
18 Siegel, L.M. and Monty, K.J. (1966) Biochim. Biophys. Acta 112, 346—362
19 Tanford, C. (1961) Physical Chemistry of Macromolecules, p. 326, John Wiley and Sons, New York
20 Laemmli, U.K. (1970) Nature 227, 680—685
21 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404—427
22 Pedrini, A.M., Dalpra, L., Ciarrocchi, G., Pedrali Noy, G.C.F., Spadari, S., Nuzzo, F. and Falaschi, A (1974) Nucleic Acids Res. 1, 193—202
23 Chan, V.L. and Ebisuzaki, K. (1970) Mol. Genet. 109, 162—168
24 Pedrali Noy, G.C.F., Dalpra, L., Pedrini, A.M., Ciarrocchi, G., Giulotto, E., Nuzzo, F. and Falaschi, A. (1974) Nucleic Acids Res. 1, 1183—1199