Gavilanes, F., Peterson, D., & Schirch, L. (1982) *J. Biol. Chem. 257*, 11431-11436.
Harruff, R. C., & Jenkins, W. T. (1976) *Arch. Biochem. Biophys. 176*, 206-213.
Jones, C. W., Hynes, J. B., & Priest, D. G. (1978) *Biochim. Biophys. Acta 524*, 55-59.
Krumdieck, C. L., & Eto, I. (1986) in *Chemistry and Biology of Pteridines* (Cooper, B. A., & Whitehead, V. M., Eds.) pp 447-466, de Gruyter, New York and Berlin.
Lu, Y.-Z., Aiello, P. D., & Matthews, R. G. (1984) *Biochemistry 23*, 6870-6876.
MacKenzie, R. E., & Baugh, C. M. (1980) *Biochim. Biophys. Acta 611*, 187-195.
Matthews, R. G., Lu, Y.-Z., Green, J. M., & MacKenzie, R. E. (1985) in *Proceedings of the Second Workshop on Folyl and Antifolyl Polyglutamates* (Goldman, D. I., Ed.) pp 65-75, Praeger, New York.
Matthews, R. G., Ross, J., Baugh, C. M., Cook, J. D., & Davis, L. (1982) *Biochemistry 21*, 1230-1238.
McGuire, J. J., & Bertino, J. R. (1981) *Mol. Cell. Biochem. 38*, 19-48.
McGuire, J. J., & Coward, J. K. (1984) in *Folates and Pterins* (Blakely, R. L., & Benkovic, S. J., Eds.) Vol. I, pp 135-190, Wiley, New York.
Priest, D. G., & Doig, M. T. (1986) *Methods Enzymol. 122*, 313-319.
Scatchard, G. (1949) *Ann. N.Y. Acad. Sci. 51*, 660.
Schirch, L. (1978) *Arch. Biochem. Biophys. 189*, 283-290.
Schirch, L., & Mason, M. (1962) *J. Biol. Chem. 237*, 2578-2581.
Schirch, L. G., & Mason, M. (1963) *J. Biol. Chem. 238*, 1032-1037.
Schirch, L., & Ropp, M. (1967) *Biochemistry 6*, 253-257.
Schirch, L., & Peterson, D. (1980) *J. Biol. Chem. 255*, 7801-7806.
Schirch, L., Tatum, C. M., & Benkovic, S. J. (1977) *Biochemistry 16*, 410-419.
Schirch, V., Hopkins, S., Villar, E., & Angelaccio, S. (1985) *J. Bacteriol. 163*, 1-7.
Scott, J. M., & Weir, D. G. (1976) *Clin. Haematol. 5*, 547-568.
Segal, I. H. (1975a) in *Enzyme Kinetics*, pp 273-329, Wiley, New York.
Segal, I. H. (1975b) in *Enzyme Kinetics*, pp 643-646, Wiley, New York.
Steenkamp, D. J., & Husain, M. (1982) *Biochem. J. 203*, 707-715.
Strong, W., Joshi, G., Lura, R., Muthukumaraswamy, N., & Schirch, V. (1987) *J. Biol. Chem. 262*, 12519-12525.
Villar, E., Schuster, B., Peterson, D., & Schirch, V. (1985) *J. Biol. Chem. 260*, 2245-2252.
Wittwer, A. J., & Wagner, C. (1981) *J. Biol. Chem. 256*, 4102-4108.
Zamierowski, M. M., & Wagner, C. (1977) *J. Biol. Chem. 252*, 933-938.

Human T-Lymphoblast Deoxycytidine Kinase: Purification and Properties†

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ABSTRACT: Previous observations present tremendous variations in the properties of deoxycytidine kinase. To clarify the properties and physiologic role of deoxycytidine kinase, we have undertaken its purification. Deoxycytidine kinase was purified from cultured human T-lymphoblasts (MOLT-4) to 90% purity with an estimated specific activity of 8 μmol min^{-1} (mg of protein)$^{-1}$. The purification procedure included ammonium sulfate precipitation, Superose-12 HPLC gel filtration chromatography, DE-52 ion-exchange chromatography, AMP–Sepharose 4B affinity chromatography, and dCTP–Sepharose-4B affinity chromatography. Deoxyguanosine, deoxyadenosine, and cytidine phosphorylating activities copurified with deoxycytidine kinase to final specific activities of 7.2, 13.5, and 4 μmol min^{-1} (mg of protein)$^{-1}$, respectively. The enzyme is very unstable at low protein concentration and is stabilized by storage at −85 °C with 1 mg/mL bovine serum albumin, 20% glycerol (v/v), 200 mM potassium chloride, and 25 mM dithiothreitol. The molecular weight was 60000, and the Stokes radius was 32 Å by gel filtration chromatography. The subunit molecular weight was 30500. This enzyme had apparent K_m values of 1.5, 430, 500, 450, and 40 μM for deoxycytidine, deoxyguanosine, deoxyadenosine, cytidine, and cytosine arabinoside, respectively. The pH optimum ranged from 6.5 to 9.0. Mg^{2+} and Mn^{2+} were the preferred divalent cations. ATP, GTP, dGTP, ITP, dITP, TTP, and XTP were substrates for the enzymes. Our study indicates that deoxycytidine kinase is a dimer with two subunits and has phosphorylating activity for deoxyguanosine, deoxyadenosine, cytidine, and cytosine arabinoside. This highly purified enzyme will facilitate the study of its regulation and phosphorylation of anticancer or antiviral nucleoside analogues.

The phosphorylation of deoxyribonucleosides to deoxyribonucleotides is a critical step in the pathogenesis of inherited immunodeficiency diseases and in the activation of specific anticancer and antiviral drugs (Carson et al., 1977, 1979; Cohen, 1966; Cohen et al., 1978a,b; Coleman et al., 1978; Gudas et al., 1978; Mejer, 1982; Mitchell et al., 1979; Ullman et al., 1978; Wortmann et al., 1979). Our previous studies have defined three distinct deoxyribonucleoside phosphorylating activities in human tissue: adenosine–deoxyadenosine

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kinase, deoxycytidine–deoxyadenosine kinase, and deoxyguanosine–deoxycytidine kinase (Hurley et al., 1983). While the properties of adenosine–deoxyadenosine kinase activity have been evaluated in detail (Hurley et al., 1985; Yamada et al., 1983, 1985), there remain many questions concerning the properties of the other activities.

Lymphoid cells are characterized by substantial quantities of nucleoside phosphorylating activities (Anderson, 1973; Gower et al., 1979; Krenitsky et al., 1976; Osborne & Scott, 1983). In S-49 lymphoma cells, deoxyguanosine kinase (dGK) activity is associated with deoxycytidine kinase (dCK) (Gudas et al., 1978), and deoxyadenosine kinase (dAK) is associated with adenosine kinase (AK) and deoxycytidine kinase (Ullman et al., 1978). In cultured human lymphoblasts, thymus, and L1210 cells, deoxyadenosine is phosphorylated by adenosine kinase and deoxycytidine kinase (Bacter et al., 1978; Carson et al., 1980; Chang et al., 1982; Durham & Ives, 1970a, 1971; Hershfield et al., 1982; Kessel, 1968; Ullman et al., 1981; Verhoef et al., 1981). These conclusions concerning substrate specificity have been derived largely from studies with mutant cell lines, and direct biochemical verification of the results has been limited by the use of partially purified or crude cell lysates which could contain more than one enzyme activity.

To clarify the properties and the physiological role of deoxycytidine kinase, we have undertaken the purification of this enzyme from cultured human lymphoblasts. Our studies show that highly purified deoxycytidine kinase is a very labile protein with multiple nucleoside phosphorylating activities.

Experimental Procedures

Materials. [5-^{3}H]Deoxycytidine, [8-^{3}H]deoxyguanosine, [8-^{3}H]deoxyadenosine, [5-^{3}H]cytidine, and [8-^{14}C]ATP were purchased from ICN Radiochemicals, Irvine, CA. Ribavirin was a gift from ICN. [2-^{14}C]Cytosine arabinoside was from Moravek Biochemicals Inc., Brea, CA. ATP, ADP, adenosine, guanosine, inosine, cytosine, thymidine, xanthosine, deoxyinosine, deoxyguanosine, deoxyadenosine, deoxyuridine, tubercidin, L-phenylisopropyladenosine, *N*-ethylcarboxamidoadenosine, 6-methylmercaptopurine riboside, aminoimidazolecarboxamide ribonucleoside, adenine arabinoside, cytosine arabinoside, *S*-adenosylhomocysteine, *S*-adenosylmethionine, 2-chloroadenosine, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, AMP–Sepharose 4B, Trizma base, bovine serum albumin, dithiothreitol, *N*-ethylmaleimide, and phenylmethanesulfonyl fluoride were purchased from Sigma Chemical Co., St. Louis, MO. Tetrahydrouridine, a cytidine deaminase inhibitor, was obtained from Calbiochem–Behring, San Diego, CA. Superose-12 (preparative grade) and 2′,3′-dideoxycytidine were purchased from Pharmacia, Piscataway, NJ. 1,10-*o*-Phenanthroline was obtained from Aldrich Chemical Co., Milwaukee, WI. Bradford reagent and low molecular weight standards for polyacrylamide gel electrophoresis were from Bio-Rad, Richmond, CA. DE-52 ion-exchange resin and Whatman 3MM chromatography paper were purchased from Fisher Scientific, Pittsburgh, PA. Low molecular weight standards for HPLC gel filtration were either from Pharmacia or from Sigma. Poly(ethylenimine)–cellulose plates were obtained from EM Science, Cherry Hills, NJ. All other reagents were of best quality as previously described (Hurley et al., 1983, 1985).

Enzyme Assay. Deoxycytidine kinase activity was assayed with a radiochemical method which quantitated the formation of the radioactively labeled nucleotide from radioactively labeled nucleoside (Hurley et al., 1983; Hurley & Fox, 1983). The incubation mixture contained 50 mM imidazole hydrochloride, pH 7.0, 2 mM ATP, 2.4 mM $MgCl_2$, 1 mg/mL bovine serum albumin, 25 mM dithiothreitol, 5% glycerol, 130 μM tetrahydrouridine, and 10 μM [5-^{3}H]deoxycytidine in a total volume of 50 μL. The enzyme was incubated at 37 °C for 5 min. The reaction rate was linear with protein concentration and with time up to 30 min. The reaction was stopped by heat inactivation for 2 min at 85 °C. After centrifugation, 20 μL of reaction mixture was spotted on DE-81 filter paper. The washing of the disks, elution of the radioactive nucleotide formed, and counting procedures were performed as previously described (Hurley & Fox, 1983). Tetrahydrouridine was added to prevent the conversion of deoxycytidine to deoxyuridine during the assay.

Deoxyguanosine and deoxyadenosine phosphorylating activities were measured under similar conditions as above except that deoxycytidine was replaced by 500 μM [8-^{3}H]deoxyguanosine or 500 μM [8-^{3}H]deoxyadenosine and the enzyme was incubated for 20 min at 37 °C. Tetrahydrouridine was also replaced either by 150 μM 8-aminoguanosine (purine-nucleoside phosphorylase inhibitor) or by 50 μM *erythro*-9-(2-hydroxy-3-nonyl)adenine (adenosine deaminase inhibitor) in the deoxyguanosine or deoxyadenosine phosphorylation assay, respectively. Cytidine or cytosine arabinoside phosphorylation was measured using 500 μM [5-^{3}H]cytidine or 500 μM [2-^{14}C]cytosine arabinoside as substrate, respectively.

In some experiments, nucleoside kinase assays were performed using 300 μM [^{14}C]ATP as radioactive substrate, and the rate of [^{14}C]ADP formation was measured. Twenty-microliter aliquots of incubation medium were spotted onto 20 × 20 cm poly(ethylenimine)–cellulose plates previously spotted with 10 mg/mL ATP and ADP as carriers. ATP was separated from ADP by thin-layer chromatography in 2 M sodium formate, pH 3.4. The carrier compounds were located with an ultraviolet light. The spots were cut out and counted with liquid scintillation cocktail by a RacBeta liquid scintillation spectrometer system. The blank value in the ADP spot was 200 dpm. There was no apparent ATPase activity.

The radiolabeled products of a [^{3}H]cytidine phosphorylation assay were identified and quantitated by high-pressure liquid chromatography. After incubation, the [^{3}H]cytidine reaction mixture was spotted onto DE-81 filter disks and washed to remove the unreacted substrate, and the reaction products were eluted with 0.5 mL of 0.2 M $NH_4H_2PO_4$. After a 10-fold dilution with water, the eluted products were loaded onto a Partisil-10 strong anion-exchange column and separated by use of a linear gradient of 5 mM $NH_4H_2PO_4$, pH 2.8, to 600 mM $NH_4H_2PO_4$, pH 3.5, over 40 min at a flow rate of 2 mL/min. Cytidine nucleotides were identified and quantitated by comparison with authentic standards. One-minute fractions were collected, and 1 mL of each fraction was placed into a scintillation vial with 0.5 mL of water and 10 mL of ACS scintillation fluid and counted in a Packard Tri-Carb liquid scintillation spectrometer.

Kinetic Studies. Apparent K_m values were determined from initial velocity studies using tritiated nucleosides. Double-reciprocal plots of velocity versus substrate concentrations were linear and used to estimate apparent K_m values. In all kinetic studies, no more than 20% of the substrate was used. The data were fit to a hyperbola with a modification of Cleland's program (Cleland, 1967) using a MicroVax II minicomputer.

All the experiments with purified deoxycytidine kinase were done 2–4 times in duplicate, and the results varied within 5–10%.

Cell Source. A human lymphoblastic cultured T-cell line, MOLT-4, either was obtained from the Massachusetts In-

stitute of Technology Cell Culture Center or was cultured and maintained in our laboratory in RPMI 1640 media supplemented with 5% donor calf serum and L-glutamine. These cells were harvested and kept frozen at −85 °C until ready to use.

Preparation of Extracts. Frozen MOLT-4 cells, 40–50 g wet weight, were allowed to thaw in 10 mM imidazole hydrochloride, pH 6.5, 5 mM dithiothreitol, 100 μM phenylmethanesulfonyl fluoride, and 100 μM *o*-phenanthroline and lysed by bomb cavitation after equilibration for 30 min at 750–1000 lb/in.^{2} of nitrogen. The buffer was adjusted to 1 g of cells/3 mL. The lysed cell extract was further homogenized using a Dounce homogenizer and spun at 48000*g* for 30 min. The supernatant was saved, and the pellet was rehomogenized with the same buffer and was spun again at 48000*g* for 30 min. The second supernatant was combined with the first supernatant with a total volume of approximately 300 mL.

Enzyme Purification. (*A*) *Ammonium Sulfate Precipitation.* The temperature was maintained at 0–4 °C throughout the purification. The protein fraction precipitating between 35 and 60% saturation with ammonium sulfate was suspended in 50 mM imidazole hydrochloride, pH 6.5, containing 2 mM ATP, 2.4 mM $MgCl_2$, 0.2 M potassium chloride, 5 mM dithiothreitol, 5% glycerol, 100 μM phenylmethanesulfonyl fluoride, and 100 μM 1,10-*o*-phenanthroline (buffer H) to a total volume of 20 mL containing approximately 800 mg of protein.

(*B*) *Gel Filtration Chromatography.* The suspended pellet was spun at 20000*g* for 5–10 min. Filtration through a nitrocellulose membrane was not possible, since it leads to a substantial loss of activity probably secondary to binding to the filter. The clarified extract was then divided into 10 2-mL fractions and applied onto a Superose-12 column (50 × 1.6 cm) which had been previously equilibrated with buffer H. The column was eluted with the same buffer at a flow rate of 1 mL/min at 300 lb/in.^{2} and a fraction size of 1 mL. The fractions containing deoxycytidine kinase activity were pooled from each injection and saved for ion-exchange chromatography. This step was performed 10 times. The void volume determination was measured using blue dextran, and the exclusion volume was determined with dinitrophenylalanine.

(*C*) *Ion-Exchange Chromatography.* Ion-exchange chromatography was performed in a 5.3 × 1.9 cm column using precycled Whatman DE-52 equilibrated with 10 mM imidazole hydrochloride, pH 6.5, 5 mM dithiothreitol, and 5% glycerol. The pooled fraction after gel filtration chromatography was diluted 4-fold with the equilibration buffer containing 100 μM phenylmethanesulfonyl fluoride, and 100 μM *o*-phenanthroline and applied to the DE-52 column. After the unbound material was collected in a batch, the column was washed with 8–10 bed volumes of the buffer containing 50 mM KCl. Next the column was eluted with a linear gradient of 100–600 mM KCl in equilibration buffer. The gradient was collected in 9-mL fractions. The peak fractions containing the enzyme activity were pooled. Glycerol sufficient to yield a 20% v/v concentration was added to the pooled fraction, which was stored at −85 °C until further processing.

(*D*) *AMP–Sepharose 4B Affinity Chromatography.* In our previous preliminary studies, deoxycytidine kinase bound to AMP–Sepharose 4B (Hurley et al., 1983). Therefore, we used this as a step of purification. 5′-AMP-Sepharose 4B was prepared for use according to the manufacturer's direction. After swelling, the gel was equilibrated with 50 mM Tris-HCl, pH 7, containing 25 mM dithiothreitol and 5% glycerol and packed in a 8.5 × 1.4 cm column. The pooled fraction from the DE-52 chromatography step was loaded following a dilution of 5-fold with the equilibrating buffer. Dilution was necessary to reduce the KCl concentration. A flow rate of 20 mL/h was controlled by a peristaltic pump, and the unbound material was collected in several batches. Ten-milliliter fractions were then collected. One hundred milliliters of the equilibrating buffer was passed through the column as a wash. The column was then eluted with 130 mL of the same buffer containing 20 μM tetrahydrouridine, followed by 130 mL of buffer with 2 mM ATP and 2.4 mM $MgCl_2$ and then with 130 mL of buffer with 2 mM ATP, 2.4 mM $MgCl_2$, and 20 μM deoxycytidine. Fifteen-microliter aliquots of each fraction together with the unbound fractions and fractions from column washing were assayed for the enzyme activity. Tetrahydrouridine, an inhibitor of cytidine deaminase, was used in an attempt to elute any of this enzyme that might be bound to the column. MgATP, a substrate for deoxycytidine kinase, was used to elute the enzyme. A wash with deoxycytidine, ATP, and Mg was used to elute enzyme that might be remaining on the column. The enzyme eluted with the MgATP wash was pooled in glycerol (20% v/v) and bovine serum albumin (1 mg/mL) to maintain activity. This pooled fraction was used for dCTP–Sepharose affinity chromatography.

(*E*) *dCTP–Sepharose 4B Affinity Chromatography.* dCTP is known to be a potent inhibitor of deoxycytidine kinase. This inhibition is reversed by TTP (Durham & Ives, 1970b; Kessel, 1968; Momparler & Fischer, 1968). We used these data to design an affinity chromatography step. dCTP–Sepharose 4B was prepared by a carbodiimide condensation procedure. Commercially available 6-aminohexanoic acid–Sepharose was washed first with 1 mM HCl and then with water. A solution of 20 mM dCTP was added to the washed gel, followed by the dropwise addition of 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide to a final concentration of 0.1 M. The gel–ligand mixture was then rotated for at least 18 h at 4 °C. The gel was washed free of unbound ligand by alternately washing with 0.1 M Tris-HCl, pH 8, and 0.1 M sodium formate, pH 5. Both buffers also contained 0.5 M sodium chloride. This procedure typically yielded 1–4 μmol of dCTP bound/mL of Sepharose.

The dCTP–Sepharose 4B column was used to purify the deoxycytidine kinase activity that had eluted from the AMP–Sepharose column. The pooled fractions of deoxycytidine kinase activity were diluted 4-fold with 50 mM Tris-HCl, pH 7.4, and 25 mM dithiothreitol to reduce the ATP and $MgCl_2$ concentrations to less than 600 μM. The diluted enzyme extract was then loaded onto a 4-mL dCTP–Sepharose column that had been equilibrated with 50 mM Tris-HCl, pH 7.4, 25 mM dithiothreitol, and 5% glycerol (buffer L). After loading of the enzyme extract, the column was washed with 40 mL of buffer L and then with 40 mL of buffer L containing 2 mM ATP and 2.4 mM $MgCl_2$ to elute nonspecifically bound proteins. Deoxycytidine kinase was eluted specifically with 20 mL of buffer L containing 100 μM deoxycytidine, 2 mM TTP, 2 mM ATP, 2.4 mM $MgCl_2$, and 200 mM KCl. Fractions were collected into 20% glycerol and assayed for enzyme activity. Fractions containing deoxycytidine kinase activity were pooled, and 1 mg/mL bovine serum albumin was added to them. This pooled fraction was stored at −85 °C for further studies. In experiments where these fractions were utilized for gel electrophoresis, no bovine serum albumin was added.

Molecular Weight Determination. The native molecular weight of deoxycytidine kinase was determined by using Superose-12 gel filtration chromatography with the column

described above. Molecular weight standards (4 mg/2 mL) were used to calibrate the column as follows: alcohol dehydrogenase (M_r 150000), bovine serum albumin (M_r 67000), ovalbumin (M_r 43000), and carbonic anhydrase (M_r 29000). The partition coefficient, K_{av}, for each standard was calculated as $(V_e - V_0)/(V_t - V_0)$, where V_e represents the elution volume of the solute and V_t and V_0 are the total volume and void volume of the column, respectively (Laurent & Killander, 1964). The estimated K_{av} was used to determine the inverse error function [$\text{Erfc}^{-1}(1 - kd)$] as described by Ackers (1967). Values for the Stokes radii of the standards were obtained from Laurent and Killander (1964) and Siegel and Monty (1965). An apparent molecular weight and Stokes radius of deoxycytidine kinase were then determined by reference to a standard curve of $\text{Erfc}^{-1}(1 - kd)$ versus the molecular weight and Stokes radius of the standard proteins.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. The subunit molecular weight and purity were determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis according to the method of Laemmli (1970). Since there was not enough protein in the final preparation to visualize the protein, the proteins were concentrated by 10–12% ice-cold trichloroacetic acid precipitation. The precipitated protein was washed 2 times using 100% ice-cold acetone. After suspension of the precipitated protein in 50 μL of H_2O, 10 μL of 100 mM dithiothreitol prepared in 10% sodium dodecyl sulfate was added and heated at 90 °C for 5 min. Then 10 μL of 100 mM *N*-ethylmaleimide was added and incubated for 15 min at room temperature to alkylate the protein (Evans et al., 1986). Sample buffer was then added, and the extract was heated further for 5 min at 95–100 °C prior to electrophoresis. Protein was applied to 1.5 × 3 cm sample wells (10% resolving gel, 4% stacking gel) and electrophoresed at 30 mA per gel for 5–6 h. The bands were visualized by silver staining according to the procedure of Wray et al. (1981). Artifactual bands originally seen on silver stain at 65–70 kDa were minimzed by reducing the β-mercaptoethanol to 2% and filtering the solutions. These bands were present even when buffer without protein was run through the precipitation, washing, denaturing, and alkylation procedures. The standards were also handled in the same manner. The molecular weight standards used were as follows: phosphorylase B (M_r 92400); bovine serum albumin (M_r 66000); ovalbumin (M_r 45000); carbonic anhydrase (M_r 30000); and soybean trypsin inhibitor (M_r 21500).

Protein Determination. Protein assays were performed with the Bradford method using bovine serum albumin as the standard (Bradford, 1976). In the final steps of purification, protein was not measurable by the Bradford method. To estimate the protein content of the purified enzyme preparation, the intensity of the protein band after silver staining was compared to the intensity of bands ranging from 10 to 100 ng of bovine serum albumin silver stained under the similar experimental condition (Codina et al., 1987). This method of assay using a densitometer is an approximation only.

Results

Purification. Deoxycytidine kinase was purified approximately 50000-fold on the basis of the protein content estimated by polyacrylamide gel electrophoresis. The estimated final specific activities of deoxycytidine kinase, deoxyguanosine kinase, and deoxyadenosine kinase are 8, 7.3, and 13.5 μmol min^{-1} mg^{-1}, respectively. The ammonium sulfate precipitation step yielded 90% of deoxycytidine kinase activity, 60% of deoxyguanosine kinase activity, and less than 50% of deoxyadenosine kinase activity (Table I).

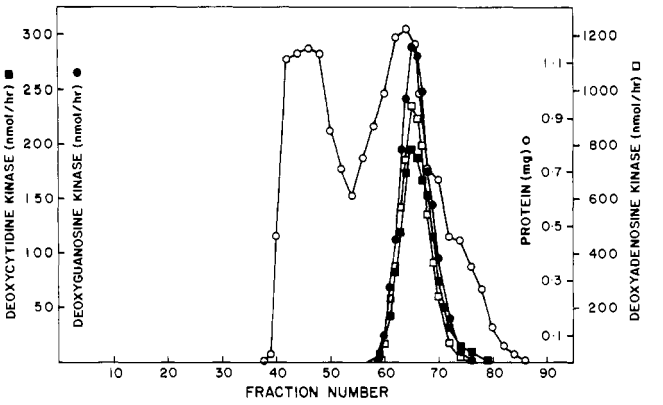

FIGURE 1: Gel filtration chromatography on Superose-12. Twenty milliliters of 35–60% ammonium sulfate saturated pellet containing 784 mg of protein was applied onto a Superose-12 HPLC gel filtration column (50 × 1.6 cm) in 2-mL aliquots. Fractions 61–73 were pooled from each injection.

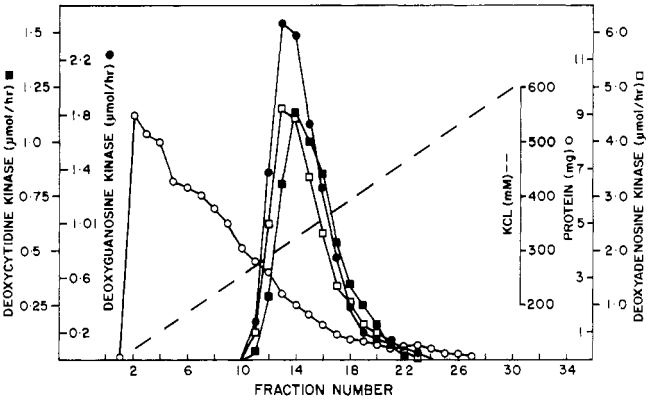

FIGURE 2: Ion-exchange chromatography on DE-52. Five hundred milliliters of a diluted Superose-12 pool containing 207 mg of protein was applied to a DE-52 column (5.3 × 1.9 cm). The column was washed as described under Experimental Procedures and eluted with a 300-mL linear KCl gradient (100–600 mM). The enzyme eluting between fractions 11 and 21 was pooled and glycerol added to 20% (v/v).

During gel filtration chromatography of deoxycytidine kinase on Superose 12, deoxyguanosine kinase and deoxyadenosine kinase activities coeluted in a single peak with deoxycytidine kinase (Figure 1). A substantial amount of protein was removed with a 7–9-fold purification of deoxycytidine kinase. The enzyme after elution from this column was relatively stable at 4 °C and retained 100% of activity at −85 °C for several weeks. However, improved stability and a significant recovery (80%) were only achieved after addition of 2 mM ATP, 2.4 mM Mg, 200 mM potassium chloride, 5 mM dithiothreitol, and 5% glycerol to the elution buffer. More than 60% of the activity was lost without the addition of these compounds. The recoveries of deoxyguanosine kinase and deoxyadenosine kinase were 57% and 24%, respectively.

A single peak of deoxycytidine kinase was eluted during ion-exchange chromatography on DE-52 cellulose (Figure 2). Addition of phenylmethanesulfonyl fluoride and *o*-phenanthroline, the protease inhibitors, dramatically shifted the elution pattern in this step from a wide plateau to a sharp peak and resulted in an increase of enzyme recovery. The recovery of deoxycytidine kinase after purification on DE-52 varied between 35 and 50% with almost 40-fold purification (Table I). However, the enzyme was very labile, and 20% glycerol (v/v) was necessary to maintain deoxycytidine kinase activity after it was eluted. It then could be stored at −85 °C for more than 12 months without the loss of activity.

Table I: Purification of Deoxycytidine Kinase

fraction	protein (mg)	deoxycytidine kinase			deoxyguanosine kinase			deoxyadenosine kinase				
		sp act.[b]	x-fold purification	recovery (%)	sp act.[b]	x-fold purification	recovery (%)	sp act.[b]	x-fold purification	recovery (%)	dCK/dGK[c]	dCK/dAK[c]
cytosol	1750	9.6	1	100	18.1	1	100	108	1	100	0.5	0.1
ammonium sulfate precipitation	784	18.6	2	87	23	1.3	60	110	1	46	0.8	0.2
gel filtration chromatography	207	65	7	80	88	5	57	198	2	24	0.8	0.3
ion-exchange chromatography	23.4	345	36	48	561	31	42	1088	10	14	0.6	0.3
AMP–Sepharose 4B chromatography	*d*			15			10			3	0.9	0.5
dCTP–Sepharose chromatography	0.001[a]	4.8×10^5	50000	4	4.3×10^5	24000	1	8×10^5	7400	0.2	1.1	0.6

[a] A rough estimate of protein content was obtained by comparing the intensity of the protein band with the standard proteins after silver staining as described under Experimental Procedures. [b] Specific activity has been expressed as nanomoles per hour per milligram of protein. dCK, deoxycytidine kinase; dGK, deoxyguanosine kinase; dAK, deoxyadenosine kinase. [c] Ratios of specific activities. [d] Protein could not be measured by the Bradford assay.

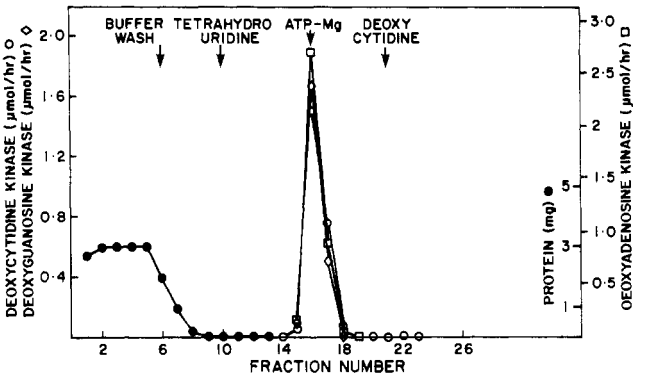

FIGURE 3: Affinity chromatography on AMP–Sepharose 4B. Five hundred milliliters of a diluted DE-52 pool containing 23.4 mg of protein was applied to an AMP–Sepharose 4B column (6.4 × 1.4 cm). Seven percent of deoxycytidine kinase did not bind to the column. The main activity peak was eluted with 2 mM ATP and 2.4 mM $MgCl_2$ (fractions 16 and 17).

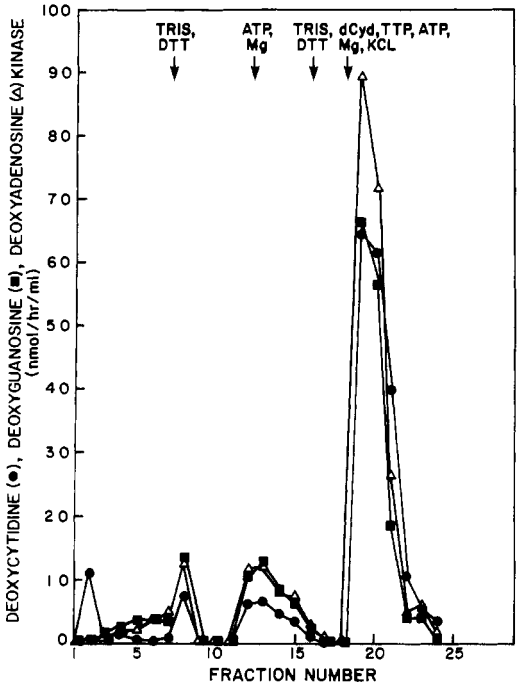

FIGURE 4: Two hundred milliliters of a diluted AMP–Sepharose pool was applied to a dCTP–Sepharose column (8.5 cm × 7 cm). Fractions 1–17 contained 10 mL each, and fractions 18–24 contained 2 mL each. The enzyme was eluted with 50 mM Tris-HCl, pH 7.4, 25 mM dithiothreitol, and 5% glycerol (peak I, fraction 8). Peak II (fractions 12–16) was eluted with 2 mM ATP and 2.4 mM $MgCl_2$, and peak III (fractions 19–24) was eluted with 100 μM deoxycytidine, 2 mM ATP, 2.4 mM $MgCl_2$, and 2 mM TTP, respectively. Peak III fractions were desalted prior to assaying for nucleoside kinase activity. DTT, dithiothreitol.

AMP-Sepharose 4B chromatography efficiently removed the majority of the remaining proteins from deoxycytidine kinase (Figure 3). However, 10–12% of deoxycytidine kinase did not bind to the column. The reapplication of this enzyme to a regenerated column did not show any further binding, suggesting that it may represent a distinct activity. About 40% of deoxycytidine kinase was eluted from this column by 2 mM ATP and 2.4 mM $MgCl_2$. This deoxycytidine kinase was associated with 10% of the starting deoxyguanosine kinase and 3% of the deoxyadenosine kinase activities (Table I).

The pooled fractions from the AMP–Sepharose 4B column that contained the deoxycytidine kinase activity were diluted and further purified on the dCTP–Sepharose 4B column

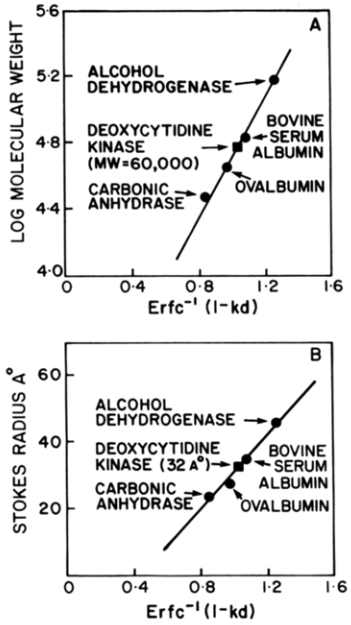

FIGURE 5: Molecular weight and Stokes radius estimates on Superose-12. The HPLC Superose-12 gel filtration column (50 × 1.6 cm) from Figure 1 was calibrated as described under Experimental Procedures. Two-milliliter samples were applied to the column. Standard proteins (4 mg) or soluble ammonium sulfate precipitate was applied to the column. Protein peaks eluted were assayed by the optical density at 280 nm or by enzyme activity as described under Experimental Procedures. (A) The log of the molecular weight is plotted against the inverse error function [$Erfc^{-1}(1 - kd)$]. (B) The Stokes radius is plotted against the inverse error function [$Erfc^{-1}(1 - kd)$].

(Figure 4). The column fractions that were eluted with buffer containing deoxycytidine, ATP, $MgCl_2$, and TTP were desalted on fresh Sephadex columns before assay to remove more than 95% of the deoxycytidine. Deoxycytidine kinase activity eluted from the dCTP–Sepharose column in three distinct peaks which contained deoxyguanosine and deoxyadenosine kinase activities. The first two peaks of deoxycytidine kinase activity accounted for less than 30% of the initial enzyme activity and did not appear to represent overloading of the column, since an enzyme preparation containing less than 6% of the activity also exhibited these two peaks of activity after purification of dCTP–Sepharose (Figure 4). The deoxycytidine kinase activity that was eluted specifically with deoxycytidine, ATP, $MgCl_2$, and TTP represented a recovery of 25% of the initial enzyme activity. Total recoveries of deoxyguanosine and deoxyadenosine kinase activities from this column were 19% and 9%, respectively. However, less than 5% of the deoxyguanosine and deoxyadenosine kinase activities were recovered in the fractions that were eluted with deoxycytidine. After purification, the increased ratios of deoxycytidine kinase to deoxyguanosine kinase or of deoxycytidine kinase to deoxyadenosine kinase suggest the removal of deoxyadenosine or deoxyguanosine phosphorylating activity that is not associated with deoxycytidine kinase (Table I).

Molecular Weight and Stokes Radius. Gel filtration chromatography indicated that the enzyme had an apparent molecular weight of 60 000 and a Stokes radius of 32 Å (Figure 5).

Subunit Molecular Weight. When the material from dCTP–Sepharose was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, it was found to consist of one major protein band that migrated with an estimated molecular weight of 30 500 (Figure 6, lanes 3–5). A small contaminating band was evident at M_r 22 000 and contained 8% of the total protein. Our gel electrophoresis demonstrated

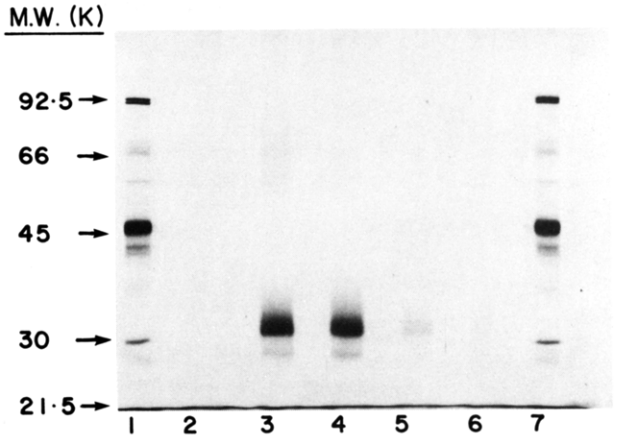

FIGURE 6: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Following dCTP–Sepharose chromatography, the fractions eluted with deoxycytidine, TTP, ATP, and $MgCl_2$ were precipitated with trichloroacetic acid and treated with dithiothreitol and *N*-ethylmaleimide before being loaded to sample wells as described under Experimental Procedures. Lanes 1 and 7 are molecular weight standards as labeled. Lane 2 corresponds to fraction 18 and does not contain any deoxycytidine kinase activity. Lanes 3, 4, and 5 correspond to fractions 19, 20, and 21, respectively (on dCTP–Sepharose chromatography; see Figure 4). Lane 6 corresponding to fraction 22 does not contain any stainable protein.

that only the M_r 30 500 band correlated with the activity of deoxycytidine kinase from the dCTP–Sepharose column, while the contaminating band did not correlate with the fractions containing deoxycytidine kinase activity. In Figure 4, fractions 19 and 20 contain the highest activity of deoxycytidine kinase, and fraction 21 had substantially less activity. The M_r 30 500 band was darkly staining in lanes 3 and 4, and this band was lightly staining in lane 5 (Figure 6). In contrast, the M_r 22 000 band was darkest staining in lane 4 and was not evident at all in lane 5. Gel electrophoresis of a less pure preparation of deoxycytidine kinase obtained by omitting the AMP–Sepharose column chromatography step showed that the M_r 22 000 band was of equal intensity to the M_r 30 500 band. In a separation purification in which the AMP–Sepharose step was added, the total activity of deoxycytidine kinase was increased which correlated with an increase in intensity of the M_r 30 500 band and a decrease in intensity of the M_r 22 000 band.

Since the native molecular weight of the enzyme is 60 000 (Figure 5), our observation suggests that deoxycytidine kinase is composed of two identical subunits.

pH Optimum. The rate of deoxycytidine phosphorylation is essentially greatest at pH 7, but the enzyme has a broad pH optimum ranging from pH 6.5 to pH 9 (Figure 7). At pH 5.5, less than 50% of the maximum rate of deoxycytidine phosphorylation was observed. Above pH 10, the activity drops substantially. The pH–activity profile of deoxyguanosine and deoxyadenosine phosphorylation showed a similar pattern.

Divalent Cation Requirement. Deoxycytidine kinase has an absolute requirement for magnesium. In the absence of added divalent cation, deoxycytidine is phosphorylated at about 10% of the rate seen with 2.4 mM Mg^{2+} (Table II). However, the addition of 200 μM EDTA removes all activity. Of the other divalent cations tested Mn^{2+}, Ca^{2+}, and Fe^{2+} allowed deoxycytidine phosphorylation to occur at about 90%, 64%, and 60% of the rate observed with $MgCl_2$, respectively. In a preliminary study, deoxycytidine was found to be phosphorylated most efficiently at equal concentrations of ATP and Mg^{2+}.

Monovalent cations, such as Na^+, K^+, and Li^+, did not substantially alter deoxycytidine kinase activity.

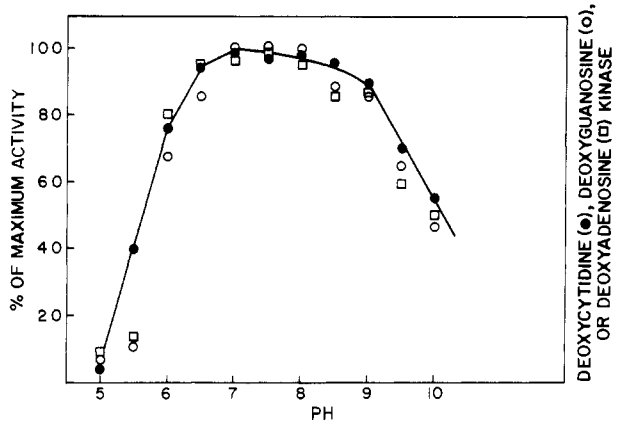

FIGURE 7: Effect of assay pH on deoxycytidine kinase activity. Different buffers were used for different pH values which are as follows: pH 4.5–6, sodium acetate; pH 6–7, imidazole hydrochloride; pH 7–10, Tris-HCl. The assay and the incubation conditions are described under Experimental Procedures. Results are expressed as the percentage of maximum deoxycytidine kinase activity which is estimated to be 8 μmol min^{-1} (mg of protein)$^{-1}$.

Table II: Divalent Cation Requirements of Deoxycytidine Kinase[a]

divalent cation (2.4 mM)	enzyme activity (%)	divalent cation (2.4 mM)	enzyme activity (%)
none	12	Fe	60
none + 200 μM EDTA	0	Mg	100
Ba	6	Mn	90
Ca	64	Hg	0
Co	27	Ni	16
Cu	2	Zn	28

[a] The pure enzyme preparation was dialyzed overnight at 4 °C against 50 mM imidazole hydrochloride, pH 7, 1 mg/mL bovine serum albumin, 5% glycerol, and 10 mM dithiothreitol. The dialyzed enzyme was preincubated with 200 μM EDTA. Each cation was tested at a final concentration of 2.4 mM. The specific activities were assayed under standard conditions as described under Experimental Procedures. Activities are expressed as a percent of enzyme activity with Mg which was 8 μmol min^{-1} (mg of protein)$^{-1}$.

Substrate Specificity. With the deoxycytidine concentration held at 0.5 μM, the concentrations of the competing nucleosides and their analogues were varied from 1 to 10 mM. Cytosine arabinoside at 2 mM caused 100% inhibition, while 2 mM deoxyguanosine, deoxyadenosine, cytidine, 2-chloroadenosine, or dideoxycytidine caused more than 40% inhibition (Table III). Uridine caused no inhibition. Using an alternative assay which quantitates the formation of radioactive ADP from ATP, we found that cytosine arabinoside, deoxyguanosine, deoxyadenosine, and cytidine are substrates for deoxycytidine kinase (Table IV). Apparent K_m values for deoxycytidine, deoxyguanosine, deoxyadenosine, cytosine arabinoside, and cytidine are 1.5, 430, 500, 40, and 450 μM, respectively. The apparent V_{max} values from double-reciprocal plots estimate the relative activity of the enzyme for these substrates. The apparent V_{max} values are as follows in micromoles per minute per milligram: deoxycytidine, 8.5; deoxyadenosine, 22; deoxyguanosine, 12; and cytidine, 7.8.

To further evaluate cytidine phosphorylation, studies were performed with 50–500 μM cytidine with and without 2 mM uridine. If deoxycytidine kinase is contaminated with uridine–cytidine kinase, cytidine phosphorylation should be inhibited by uridine. We did not see any inhibition of cytidine phosphorylation by uridine (data not shown). The phosphorylation of radioactive cytidine to radioactive CMP was confirmed by coelution of the radioactivity with authentic CMP during high-performance liquid chromatography. During the purification, cytidine kinase activity coeluted with deoxycytidine kinase. However, 98% of the relative cytidine kinase activity was removed (Table V).

Guanosine triphosphate was as effective a phosphate donor as ATP (Table VI). dGTP, ITP, dITP, and XTP had more than 80% of activity as compared to ATP.

Enzyme Stability. The important stabilizing factor for the purified deoxycytidine kinase was found to be the protein concentration and addition of glycerol and dithiothreitol throughout the purification procedure. In addition to 1 mg/mL bovine serum albumin, 25 mM dithiothreitol and 20% glycerol, 2 mM ATP, 2.4 mM $MgCl_2$, and 200 mM potassium chloride were found to stabilize the enzyme activity over a longer period of time at −85 °C. Even with the addition of all these compounds, the enzyme is less stable at 4 °C. Filtration of this enzyme through a nitrocellulose membrane was associated with a 40% decrease of enzyme activity probably due to the binding of the enzyme protein to the filter, while the enzyme was only partially purified. Passage of highly purified enzyme through such filters leads to a complete loss of enzyme activity.

Multiple Peaks of Activity. In order to determine whether the different peaks of deoxycytidine kinase eluted from the dCTP–Sepharose column (Figure 4) were distinctive activities, we have compared apparent K_m values of deoxycytidine, deoxyguanosine, and deoxyadenosine for the enzymes in three different peaks. The enzymes of peak I and peak II were concentrated as follows: 10 mL of peak I or 59 mL of peak II was loaded onto two separate 1-mL DE-52 columns, the columns were washed with 10 mL of buffer L containing 1 mg/mL bovine serum albumin, and the enzymes were eluted in 2–3 mL of the same buffer containing 1 mg/mL bovine serum albumin and 500 mM potassium chloride. The apparent K_m values were found to be very similar for all three enzyme

Table III: Inhibition of Deoxycytidine Phosphorylation by Nucleosides[a]

compound (2 mM)	inhibition (%)	compound (2 mM)	inhibition (%)
adenosine	14	L-phenylisopropyladenosine	22
guanosine	10	*N*-ethylcarboxamidoadenosine	22
inosine	5	6-methylmercaptopurine riboside	15
cytidine	44	aminoimidazolecarboxamide ribonucleoside	14
thymidine	5	adenine arabinoside	14
uridine	0	cytosine arabinoside	100
xanthosine	0	*S*-adenosylhomocysteine	0
deoxyinosine	14	*S*-adenosylmethionine	5
deoxyguanosine	40	2-chloroadenosine	53
deoxyadenosine	48	ribavirin	11
deoxyuridine	14	dideoxycytidine	54
tubercidin	18		

[a] The inhibition of deoxycytidine kinase by different nucleosides was tested with 0.5 μM deoxycytidine under standard assay conditions. The estimated activity with no additional nucleoside was 5.5 μmol min^{-1} (mg of protein)$^{-1}$.

Table IV: Substrate Specificity of Deoxycytidine Kinase[a]

compound (2 mM)	enzyme activity (%)	compound (2 mM)	enzyme activity (%)
deoxycytidine	100	6-methylmercapto-purine riboside	0
cytidine	70	aminoimidazolecarbox-amide ribonucleoside	0
deoxyguanosine	117	L-phenylisopropyl-adenosine	12
deoxyadenosine	136	*N*-ethylcarboxamido-adenosine	0
cytosine arabinoside	93	dideoxycytidine	5
adenine arabinoside	11		
uridine	0		
2-chloroadenosine	7		

[a] With different nucleosides at 2 mM concentration, the substrate specificity of deoxycytidine kinase was tested by measuring [^{14}C]ADP formation from [^{14}C]ATP as described under Experimental Procedures. Results have been expressed as the percentage of activity with 2 mM deoxycytidine which was 7.7 μmol min^{-1} (mg of protein)$^{-1}$.

Table V: Copurification of Cytidine Kinase with Deoxycytidine Kinase[a]

fraction	sp act. (nmol h^{-1} mg^{-1})	*x*-fold purification	recovery (%)	dCK/CK[b]
cytosol	198	1	100	0.05
ammonium sulfate precipitation	363	1.8	82	0.05
gel filtration chromatography	248	1.25	15	0.26
ion-exchange chromatography	606	3.0	4.0	0.6
AMP–Sepharose chromatography				
dCTP–Sepharose chromatography	2.5×10^5	1262	0.07	2.0

[a] The purification of cytidine kinase was estimated by assaying the fractions described in Table I. [b] Ratio of specific activities of deoxycytidine kinase (dCK) to cytidine kinase (CK).

Table VI: Phosphate Donor Specificity of Deoxycytidine Kinase[a]

phosphate donor (2 mM)	enzyme activity (%)	phosphate donor (2 mM)	enzyme activity (%)
ATP	100	dCTP	0
dATP	40	ITP	100
ADP	7	dITP	83
dADP	0	UTP	14
GTP	114	dUTP	25
dGTP	93	TTP	70
araGTP	107	XTP	105
CTP	13	ZTP[b]	96

[a] ATP present in the purified enzyme preparation was removed by dialysis against 50 mM imidazole hydrochloride, pH 7, 1 mg/mL bovine serum albumin, 5% glycerol, and 10 mM dithiothreitol for 17 h at 4 °C. The ability of nucleotides to substitute for ATP as phosphate donor was assessed by incubating the dialyzed enzyme with 2 mM nucleotide under standard assay conditions as described under Experimental Procedures. Activities are expressed as percent of enzyme activity with 2 mM ATP which was 7 μmol min^{-1} (mg of protein)$^{-1}$. Several nucleoside diphosphates and their deoxy compounds, such as GDP, CDP, IDP, UDP, dCDP, dGDP, and dIDP, could not substitute for ATP as phosphate donor. [b] Aminoimidazolecarboxamide ribofuranosyl triphosphate.

fractions which were 1.0–1.5 μM for deoxycytidine, 430–490 μM for deoxyguanosine, and 410–500 μM for deoxyadenosine. There was no substantial difference in the pH optimum (Figure 7). These data suggest that deoxycytidine–deoxyguanosine–deoxyadenosine kinase activities eluted from the dCTP–Sepharose column in three separate peaks are the same enzyme. The first two peaks may have resulted from changes to buffer indicated in Figure 4.

Discussion

Nucleoside kinase activities have several functions. Ribonucleoside and deoxyribonucleoside kinases have a salvage activity (Cooper & Perry, 1966; Grav, 1967) by phosphorylating nucleosides to their respective nucleotide derivatives. However, their activity may lead to the synthesis of compounds toxic to cells in malignancy and in specific immune deficiency disease states (Anderson, 1973; Bennet et al., 1973, 1978; Bennett & Hill, 1975; Carson et al., 1977; Divekar & Hikala, 1971; Parks & Brown, 1973). Finally, they phosphorylate nucleoside analogues with antiviral or anticancer activities. Therefore, the purification and identification of the kinases responsible for the phosphorylation of each nucleoside are essential in order to clarify the specific functions of these enzymes.

Deoxycytidine kinase has been partially purified from many sources (Baxter et al., 1978; Bohman & Eriksson, 1985; Cheng et al., 1977; Durham & Ives, 1970a,b; Kozai et al., 1972; Krenitsky et al., 1976; Meyers & Kreis, 1976, 1978; Mollgaard, 1980; Momparler & Fischer, 1968; Sarup & Fridland, 1987; Szyfter et al., 1985) including the following human tissues: tonsil lymphocytes, leukemia spleen, T-lymphoblasts, and myeloblasts. The enzyme is located in the cytoplasm of the cell in our study and other reports. The significance of the reported mitochondrial enzyme is unclear (Cheng et al., 1977). We have purified deoxycytidine kinase to 90% purity from human cultured T-lymphoblasts to an estimated specific activity of 8 μmol min^{-1} mg^{-1}. This is the highest purification reported to date from human cells. Other purifications from mammalian tissue have obtained specific activities of 0.005–0.10 μmol min^{-1} mg^{-1} (Bohman & Eriksson, 1985; Durham & Ives, 1970; Kessel, 1968; Krenitsky et al., 1976; Meyers & Kreis, 1976; Momparler & Fischer, 1968; Sarup & Fridland, 1987; Szyfter et al., 1985). Our final preparation represents a greater than 50 000-fold purification over the original supernatant and results in a single major band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. In contrast, the previous best purification reported was 1800-fold, resulting in 10% purity. No subunit molecular weight was determined on this preparation (Sarup & Fridland, 1987). The two critical aspects of our preparation were the elution of deoxycytidine kinase from dCTP–Sepharose and the maintenance of a high protein concentration and 20% glycerol to ensure stability of the enzyme.

The molecular weight of native deoxycytidine kinase was estimated to be 60 000. This value is similar to that for deoxycytidine kinase from human leukemic spleen (Bohman & Eriksson, 1985), calf thymus (Durham & Ives, 1970a), and L1210 cells (Kessel, 1968) and in the range of 47 000–68 000 previously observed for deoxycytidine kinase from *Bacillus subtilis*, human myeloblasts, *Lactobacillus acidophilus*, and calf thymus (Diebel & Ives, 1977; Krygier & Momparler, 1971; Martin & Ives, 1978; Meyers & Kreis, 1976; Mollgaard, 1980). With a subunit molecular weight of 30 500, the enzyme appears to be a dimer consisting of two identical subunits (Figure 6). A similar subunit molecular weight was suggested by correlating a specific band with enzyme activity in a partially purified preparation from leukemic spleen (Bohamn & Eriksson, 1985).

The highly purified enzyme has some properties that are similar to partially purified preparations. The broad plateau of activities between pH 6.5 and 9 for human T-lymphoblast deoxycytidine kinase (Figure 7) resembles that reported for the enzyme from calf thymus (Durham & Ives, 1970a) and from acute lymphoblastic and chronic myelocytic leukemia

cells (Sarup & Fridland, 1987). In addition, the absolute requirement for divalent metal ions and the specificity were similar to enzymes from other sources (Durham & Ives, 1970a; Sarup & Fridland, 1987). Deoxycytidine kinase has a broad specificity for phosphate donors (Table VI). Although previous reports showed that GTP and dGTP are the most efficient phosphate donors (Momparler & Fischer, 1968; Mollgaard, 1980), we found ATP was equally efficient (Table VI).

Deoxycytidine, deoxyguanosine, and deoxyadenosine phosphorylating activities coeluted during gel filtration, ion-exchange chromatography, and two affinity chromatography columns (Figures 1–4). Previous observations present tremendous variations in the apparent substrate specificity of deoxycytidine kinases. This is most probably related to the study of partially purified enzymes which may contain more than one active protein and tissue-specific differences. In P815 murine neoplastic cells, deoxyguanosine kinase was completely separated from deoxycytidine kinase (Meyers & Kreis, 1976). Deoxycytidine kinases in continuous cell lines, such as S-49 lymphoma cells, WIL-2, CCRF-CEM, and L1210 cells, and thymus phosphorylate deoxyadenosine (Carson et al., 1977, 1980; Hershfield et al., 1982; Ullman et al., 1978, 1981; Verhoef et al., 1981) or deoxyguanosine (Gudas et al., 1978; Verhoef et al., 1981). A highly purified bacterial enzyme phosphorylates deoxyadenosine (Mollgaard, 1980). Calf thymus soluble deoxycytidine kinase phosphorylates deoxyguanosine and deoxyadenosine (Durham & Ives, 1970a; Ives & Durham, 1970; Gower et al., 1979; Krenitsky et al., 1976; Momparler & Fischer, 1968), while the human thymus enzyme phosphorylates deoxyadenosine (Carson et al., 1977). Human leukemic T-lymphoblasts and myeloblasts possess deoxycytidine kinase that contains the major phosphorylating activity for deoxyadenosine and deoxyguanosine (Sarup & Fridland, 1987). T-Lymphoblast-specific nucleoside kinases from MOLT 4F, CCRF-CEM, and RPM1 8402 cell extracts have a high specificity for deoxycytidine, deoxyguanosine, and deoxyadenosine phosphorylation (Yamada et al., 1983). This T-lymphoblast-specific nucleoside kinase may be different from deoxycytidine kinase (Yamada et al., 1985) in MOLT-4 cell extracts and was not found by us using MOLT-4 cell lines or by others using CCRF-CEM cell lines (Sarup & Fridland, 1987).

The apparent K_m values for MOLT-4 deoxycytidine kinase are 1.5, 430, and 500 μM for deoxycytidine, deoxyguanosine, and deoxyadenosine, respectively. These K_m values are within the range reported by others: 0.3–50 μM for deoxycytidine, 0.1–1.0 mM for deoxyadenosine, and 0.1–3.0 mM for deoxyguanosine (Carson et al., 1977; Durham & Ives, 1970a; Ives & Durham, 1970; Kessel, 1968; Kozai et al., 1972; Krenitsky et al., 1976; Martin & Ives, 1978; Momparler & Fischer, 1968; Sarup & Fridland, 1987; Szyfter et al., 1985; Yamada et al., 1983). The activation observed with nucleoside substrates in partially purified deoxycytidine kinase (Sarup & Fridland, 1987) was not evident in our highly purified preparation.

Phosphorylation of cytidine by partially purified deoxycytidine kinase has been a controversial matter (Durham & Ives, 1970a; Krenitsky et al., 1976; Krygier & Momparler, 1971; Momparler & Fischer, 1968). In partially purified L1210 cells, deoxycytidine, deoxyguanosine, deoxyadenosine, and cytidine phosphorylation is associated with the same deoxynucleoside kinase (Chang et al., 1982). Our studies revealed definite cytidine phosphorylating activity associated with the highly purified deoxycytidine kinase. This activity represents 2% of the relative starting cytidine kinase, indicating the removal of most of this activity. This phosphorylation is unlikely to be due to contamination with cytidine–uridine kinase, since uridine is not a substrate for our enzyme preparation (Table IV). Moreover, the K_m (450 μM) for cytidine phosphorylation with our enzyme is 3–10-fold higher than the reported K_m values for cytidine–uridine kinase (Ahmed & Baker, 1980; Payne et al., 1985). We conclude that cytidine phosphorylation is a property of highly purified deoxycytidine kinase.

The substrate specificity for deoxycytidine kinase includes a variety of nucleoside analogues with therapeutic efficacy in humans (Durham & Ives, 1970a, 1971; Kozai, et al., 1972; Momparler & Fischer, 1968; Nakai & LePage, 1972). This enzyme has been reported to be the rate-limiting step in the activation of the chemotherapeutic agent cytosine arabinoside to its 5′-triphosphate (Cohen, 1966; Mejer, 1982). Consistent with that finding, our studies demonstrate that cytosine arabinoside is a substrate for highly purified deoxycytidine kinase. Adenine arabinoside, an antiviral drug, is phosphorylated by adenosine kinase and deoxycytidine kinase (Cozzarelli, 1977; Henderson et al., 1980; Plunkett et al., 1980; Sarup & Fridland, 1987; Yamada et al., 1985). In our studies, this compound has minor if any activity with MOLT-4 deoxycytidine kinase. 2′,3′-Dideoxycytidine, which is active against human immunodeficiency virus (Cate & Cheng, 1987), is not clearly a substrate for our purified enzyme or has a very low V_{max} (Table III). This latter compound is a poor substrate for partially purified human T-lymphoblastic cytoplasmic and mitochondrial deoxycytidine kinases (Cate & Cheng, 1987). Studies with a radioactive derivative are necessary to clarify this matter.

The existence of highly purified human T-lymphoblastic deoxycytidine kinase will facilitate our delineation of the role for this enzyme in nucleoside and nucleoside analogue phosphorylation. With this method, it should be possible to purify similar activities from other tissues to compare their properties. This is important for the development of nucleoside analogues with specific anticancer or antiviral activities.

ACKNOWLEDGMENTS

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Registry No. dCK, 9039-45-6; deoxycytidine, 951-77-9; deoxyguanosine, 961-07-9; deoxyadenosine, 958-09-8; cytidine, 65-46-3; cytosine arabinoside, 147-94-4; magnesium, 7439-95-4.

REFERENCES

Ackers, G. K. (1967) *J. Biol. Chem. 242*, 3237–3238.

Ahmed, N. K., & Baker, D. R. (1980) *Cancer Res. 40*, 3559–3563.

Anderson, E. P. (1973) *Enzymes (3rd Ed.) 9*, 46–96.

Baxter, A., Currie, L. M., & Durham, J. P. (1978) *Biochem. J. 173*, 1005–1008.

Bennett, L. L., Jr., & Hill, D. L. (1975) *Mol. Pharmacol. 11*, 803–808.

Bennett, L. L., Jr., Vail, M. H., Allan, P. W., & Shaddix, S. C. (1973) *Biochem. Pharmacol. 22*, 1221–1227.

Bennett, L. L., Jr., Hill, D. L., & Allan, P. W. (1978) *Biochem. Pharmacol. 27*, 83–87.

Bohman, C., & Eriksson, S. (1985) *Adv. Exp. Med. Biol. 195B*, 311–314.

Bradford, M. (1976) *Anal. Biochem. 72*, 248–252.

Carson, D. A., Kaye, J., & Seegmiller, J. E. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5677–5681.

Carson, D. A., Kaye, J., Matsumoto, J. E., Seegmiller, J. E., & Thompson, L. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2430–2433.

Carson, D. A., Wasson, D. B., Kaye, J., Ullman, B., Martin, D. W., Jr., Robins, R., & Montgomery, J. A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6865–6869.
Cate, M., & Cheng, Y. (1987) *J. Biol. Chem. 262*, 988–991.
Chang, C. H., Brockman, R. W., & Bennett, L. L., Jr. (1982) *Cancer Res. 42*, 3033–3039.
Cheng, Y. C., Domin, B., & Lee, L. S. (1977) *Biochim. Biophys. Acta 481*, 481–492.
Cleland, W. W. (1967) *Adv. Enzymol. Relat. Areas Mol. Biol. 25*, 1–32.
Codina, J., Yatari, A., Grenet, D., Brown, A. M., & Birnbaumer, L. 1987) *Science (Washington, D.C.) 236*, 442–445.
Cohen, A., Gudas, L. J., Ammann, A. J., Stall, G. E. J., & Martin, D. W., Jr. (1978a) *J. Clin. Invest. 61*, 1405–1409.
Cohen, A., Hirschhorn, R., Horowitz, S., Rubinstein, A., Polmar, S. H., Hong, R., & Martin, D. W., Jr. (1978b) *Proc. Natl. Acad. Sci. U.S.A. 75*, 472–476.
Cohen, S. S. (1966) *Prog. Nucleic Acid Res. Mol. Biol. 5*, 1–88.
Coleman, M. S., Donafrio, J., Hutton, J. J., Hahn, L., Daoud, A., Lampkin, B., & Dyminski, J. (1978) *J. Biol. Chem. 253*, 1619–1626.
Cooper, R. A., Perry, S., & Breitman, T. R. (1966) *Cancer Res. 26*, 2267–2275.
Cozzarelli, N. R. (1977) *Annu. Rev. Biochem. 46*, 641–648.
Diebel, M. R., Jr., & Ives, D. H. (1977) *J. Biol. Chem. 252*, 8235–8239.
Divekar, A. Y., & Hikala, M. T. (1971) *Mol. Pharmacol. 7*, 663–673.
Durham, J. P., & Ives, D. H. (1970a) *J. Biol. Chem. 245*, 2276–2284.
Durham, J. P., & Ives, D. H. (1970b) *J. Biol. Chem. 245*, 2285–2294.
Durham, J. P., & Ives, D. H. (1971) *Biochim. Biophys. Acta 228*, 9–25.
Evans, T., Brown, M. L., Fraser, E. D., & Northup, J. K. (1986) *J. Biol. Chem. 261*, 7052–7059.
Gower, W. R., Carr, M. C., & Ives, D. H. (1979) *J. Biol. Chem. 254*, 2181–2183.
Grav, H. J. (1967) in *Methods in Cancer Research* (Busch, H., Ed.) Vol. 3, pp 243–398, Academic Press, New York.
Gudas, L. J., Ullman, B., Cohen, A., & Martin, D. W., Jr. (1978) *Cell (Cambridge, Mass.) 14*, 531–538.
Henderson, J. F., Scott, F. W., & Lowe, J. K. (1980) *Pharm. Ther. 8*, 573–604.
Hershfield, M. S., Fetter, J. E., Small, W. C., Bagnara, A. S., Williams, S. R., Ullman, B., Martin, D. W., Jr., Wasson, D. B., & Carson, D. A. (1982) *J. Biol. Chem. 257*, 6380–6386.
Hurley, M. C., & Fox, I. H. (1983) *Biochem. Med. 30*, 89–100.
Hurley, M. C., Palella, T. D., & Fox, I. H. (1983) *J. Biol. Chem. 258*, 15021–15027.
Hurley, M. C., Lin, B., & Fox, I. H. (1985) *J. Biol. Chem. 260*, 15675–15681.
Ives, D. H., & Durham, J. P. (1970) *J. Biol. Chem. 245*, 2285–2294.
Kessel, D. (1968) *J. Biol. Chem. 243*, 4739–4744.
Kozai, Y., Sonada, S., Kobayashi, S., & Sugino, Y. (1972) *J. Biochem. (Tokyo) 71*, 485–496.
Krenitsky, T. A., Tuttle, J. V., Koszalka, G. W., Chen, I. S., Beacham, L. M., III, Rideout, J. L., & Elion, G. B. (1976) *J. Biol. Chem. 251*, 4055–4061.
Krygier, V., & Momparler, R. L. (1971) *J. Biol. Chem. 246*, 2745–2751.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–686.
Laurent, T. C., & Killander, J. (1964) *J. Chromatog. 14*, 317–330.
Martin, R. D., Jr., & Ives, D. H. (1978) *Methods Enzymol. 51*, 346–354.
Mejer, J. (1982) *Scand. J. Clin. Lab. Invest. 42*, 401–406.
Meyers, M. B., & Kreis, W. (1976) *Arch. Biochem. Biophys. 177*, 10–15.
Meyers, M. B., & Kreis, W. (1978) *Cancer Res. 38*, 1105–1112.
Mitchell, B. S., Mejias, E., Daddona, P., & Kelley, W. N. (1979) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5011–5014.
Mollgaard, H. (1980) *J. Biol. Chem. 255*, 8216–8220.
Momparler, R. L., & Fischer, G. A. (1968) *J. Biol. Chem. 243*, 4298–4304.
Nakai, Y., & LePage, G. A. (1972) *Cancer Res. 32*, 2445–2451.
Osborne, W. R. A., & Scott, C. R. (1983) *Biochem. J. 214*, 711–718.
Parks, R. E., Jr., & Brown, P. R. (1973) *Biochemistry 12*, 3294–3302.
Payne, R. C., Cheng, N., & Traut, T. W. (1985) *J. Biol. Chem. 260*, 10242–10247.
Plunkett, W., Chubb, S., Alexander, L., & Montgomery, J. A. (1980) *Cancer Res. 40*, 2349–2355.
Sarup, J. C., & Fridland, A. (1987) *Biochemistry 26*, 590–597.
Siegel, L., & Monty, K. J. (1965) *Biochem. Biophys. Res. Commun. 19*, 494–499.
Szyfter, K., Sasvari-Szekely, M., Spasokukotskaja, T., Antoni, F., & Staub, M. (1985) *Acta Biochim. Biophys. Acad. Sci. Hung. 20*, 173–182.
Ullman, B., Gudas, L. J., Cohen, A., & Martin, D. W., Jr. (1978) *Cell (Cambridge, Mass.) 14*, 365–375.
Ullman, B., Levinson, B. B., Hershfield, M. S., & Martin, D. W., Jr. (1981) *J. Biol. Chem. 256*, 848–852.
Verhoef, V., Sarup, J., & Fridland, A. (1981) *Cancer Res. 41*, 4478–4483.
Wortmann, R. L., Andres, C. M., Kaminska, J., & Fox, I. H. (1979) *Arthritis Rheum. 22*, 524–531.
Wray, W., Boulikas, T., Wray, V. P., & Hancock, R. (1981) *Anal. Biochem. 118*, 197–203.
Yamada, Y., Goto, H., & Ogasawara, N. (1983) *Biochim. Biophys. Acta 761*, 34–40.
Yamada, Y., Goto, H., & Ogasawara, N. (1985) *Int. J. Biochem. 17*, 425–428.