Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 1531–1535, February 1991
Medical Sciences

Cloning and expression of human deoxycytidine kinase cDNA

(deoxyribonucleosides/T lymphoblasts/immunodeficiency/cytosine arabinoside)

E. G. CHOTTINER*, D. S. SHEWACH†, N. S. DATTA*, E. ASHCRAFT*, D. GRIBBIN*, D. GINSBURG*‡§, I. H. FOX*¶, AND B. S. MITCHELL*†‖

Departments of *Internal Medicine, †Pharmacology, and §Human Genetics, and ‡Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109

Communicated by Eloise R. Giblett, November 26, 1990 (received for review September 24, 1990)

ABSTRACT **Deoxycytidine (dCyd) kinase is required for the phosphorylation of several deoxyribonucleosides and certain nucleoside analogs widely employed as antiviral and chemotherapeutic agents. Detailed analysis of this enzyme has been limited, however, by its low abundance and instability. Using oligonucleotides based on primary amino acid sequence derived from purified dCyd kinase, we have screened T-lymphoblast cDNA libraries and identified a cDNA sequence that encodes a 30.5-kDa protein corresponding to the subunit molecular mass of the purified protein. Expression of the cDNA in *Escherichia coli* results in a 40-fold increase in dCyd kinase activity over control levels. In dCyd kinase-deficient murine L cells, transfection with dCyd kinase cDNA in a mammalian expression vector produces a 400-fold increase over control in dCyd phosphorylating activity. The expressed enzyme has an apparent K_m of 1.0 μM for dCyd and is also capable of phosphorylating dAdo and dGuo. Northern blot analysis reveals a single 2.8-kilobase mRNA expressed in T lymphoblasts at 5- to 10-fold higher levels than in B lymphoblasts, and decreased dCyd kinase mRNA levels are present in T-lymphoblast cell lines resistant to arabinofuranosylcytosine and dideoxycytidine. These findings document that this cDNA encodes the T-lymphoblast dCyd kinase responsible for the phosphorylation of dAdo and dGuo as well as dCyd and arabinofuranosylcytosine.**

Deoxycytidine (dCyd) kinase (NTP:deoxycytidine 5′-phosphotransferase; EC 2.7.1.74) is responsible for the phosphorylation of several deoxyribonucleosides and their analogs. The enzyme has been shown to have broad substrate specificity for dAdo and dGuo as well as for dCyd (1–7) and plays a physiologic role in the maintenance of normal deoxyribonucleotide pools. dCyd kinase is also a key enzyme in the phosphorylation of a variety of antineoplastic and antiviral nucleoside analogs including 1-β-D-arabinofuranosylcytosine ("cytosine arabinoside," araC) (8, 9) and dideoxycytidine (ddCyd) (10), and deficiency of dCyd kinase activity mediates resistance to these drugs. The enzyme is allosterically regulated by several deoxyribonucleotides and preferentially uses ATP as a phosphate donor for the phosphorylation of dCyd (1–7, 11). A more detailed understanding of the structure of this enzyme could therefore be of use in the design of new chemotherapeutic agents.

In comparison with other normal and leukemic lymphoid cells, human and murine thymocytes and T lymphoblasts have relatively high levels of dCyd kinase activity (12–14), and it has been postulated that this increased enzyme activity plays a role in the sensitivity of these T cells to deoxyribonucleoside-induced cytotoxicity (15). However, it has not been determined whether the variability in activity is due to alterations at the level of gene expression. In order to define the structure and regulation of dCyd kinase, we screened a MOLT-4 T-lymphoblast cDNA library with an oligonucleotide probe coding for a 12-amino acid peptide sequence obtained from purified protein. We have obtained a nearly full-length cDNA clone** encoding a 30.5-kDa protein that, when expressed in mammalian cells, has the kinetic properties and substrate specificity of the purified dCyd kinase protein. This sequence bears no relation to a previously reported dCyd kinase clone (16).

MATERIALS AND METHODS

Cell Lines. Human T-lymphoblast (MOLT-4, Jurkat) and B-lymphoblast (MGL-8, GM558) cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. Three cell lines deficient in dCyd kinase activity, including a murine L-cell line and two human CCRF-CEM T-lymphoblast lines selected in either araC or ddCyd, were graciously provided by Buddy Ullman. The parent cell line AB9228, deficient in hypoxanthine phosphoribosyltransferase (HPRT) activity, was mutagenized with *N*-methyl-*N*-nitroso-*N*′-nitroguanidine (3 μg/ml). The dCyd kinase-deficient clone Ara-C-8D was selected in 8 μM araC (10, 17), whereas the ddCyd-resistant clone was selected in 50 μM ddCyd.

Purification and Amino Acid Sequencing of dCyd Kinase Protein. dCyd kinase was purified as described (7) from T lymphoblasts obtained at the time of therapeutic leukapheresis of a patient with T-cell acute lymphoblastic leukemia. Fractions containing the majority of dCyd kinase activity were eluted from a dCTP-Sepharose column and desalted and concentrated on a C_8 microbore column by using a stepwise gradient from 100% solvent A (0.1% trifluoroacetic acid) to 100% solvent B (acetonitrile/2-propanol/water, 60:15:15, vol/vol). Fractions corresponding to a single major peak of 30.5 kDa were pooled, the protein was digested by trypsin, and the peptides were separated by reverse-phase HPLC. Amino acid sequence analysis was performed by the Yale University protein sequencing facility under the direction of Ken Williams.

Isolation and Characterization of cDNA Clones. A MOLT-4 cDNA library constructed in λgt11 as described (18) was screened with a ^{32}P-end-labeled 35-base oligonucleotide coding for a 12-amino acid sequence obtained from tryptic digests of the purified protein. Hybridizations were carried out at 42°C in 5× SSC/10 mM NaH_2PO_4/2.5 mM EDTA/1×

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: araC, 1-β-D-arabinofuranosylcytosine; ddCyd, dideoxycytidine; HPRT, hypoxanthine phosphoribosyltransferase.

¶Present address: Biogen, 14 Cambridge Center, Cambridge, MA 02142.
‖To whom reprint requests should be addressed at: University of Michigan, 1150 West Medical Center Drive, 5510 Medical Science Research Building I, Ann Arbor, MI 48109-0680.

**The sequence reported in this paper has been deposited in the GenBank data base (accession no. M60527).

Denhardt's solution containing herring testis DNA at 150 μg/ml. (SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7; Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin.) Filters were washed at 37°C in 2× SSC/0.1% SDS and autoradiographed for 72 hr at −70°C. λ phage from positive plaques were purified and the cDNA inserts were subcloned into the pGEM-4Z vector (Promega) for sequencing. A 2.2-kilobase (kb) cDNA clone isolated from this library contained a nucleotide sequence that encoded the 12-amino acid segment and was used to screen a CCRF-CEM library constructed in a modified Okayama–Berg expression vector (19). Two additional clones with inserts of 2.2 and 2.5 kb (pDCK3B1A) were isolated from this library and sequenced by the Sanger dideoxynucleotide method (20) using synthetic oligonucleotide primers complementary to each strand of the cDNA. Both strands were sequenced in their entirety from one or more independent clones. Data were analyzed using European Molecular Biology Laboratory (EMBL), GenBank, and National Biomedical Research Foundation (NBRF) databases and the Genetics Computer Group package (Madison, WI).

RNA Extraction and Northern Blot Analysis. RNA was prepared by guanidine hydrochloride extraction and precipitation (21), electrophoresed in denaturing formaldehyde/agarose gels, and transferred to nitrocellulose by standard methods (22). Single-stranded RNA standards (BRL) were used for size determinations. Blots were hybridized at 42°C in 50% (vol/vol) formamide/10% (wt/vol) dextran sulfate/5× SSPE/1× Denhardt's solution/0.1% SDS containing herring testis DNA at 150 μg/ml. (SSPE is 0.15 M NaCl/0.01 M sodium phosphate, pH 7.4/1 mM EDTA.) dCyd kinase and actin (23) cDNA probes were labeled by the random hexamer labeling technique (24). Blots were washed

```
AAAGTCAAACCCCGACACCGCGGCGGGCCGGTGAGCTCACTAGCTGACCCGGCAGGTCAGGATCTGGCTTAGCGGCGCCGCGAGCTCCAGTGCGCGCACCCGTG   104
GCCGCCTCCCAGCCCTCTTTGCCGGACGAGCTCTGGGCCGCCACAAGACTAAGGA ATG GCC ACC CCG CCC AAG AGA AGC TGC CCG TCT TTC   196
                                                          Met Ala Thr Pro Pro Lys Arg Ser Cys Pro Ser Phe

TCA GCC AGC TCT GAG GGG ACC CGC ATC AAG AAA ATC TCC ATC GAA GGG AAC ATC GCT GCA GGG AAG TCA ACA TTT GTG   273
Ser Ala Ser Ser Glu Gly Thr Arg Ile Lys Lys Ile Ser Ile Glu Gly Asn Ile Ala Ala Gly Lys Ser Thr Phe Val

AAT ATC CTT AAA CAA TTG TGT GAA GAT TGG GAA GTG GTT CCT GAA CCT GTT GCC AGA TGG TGC AAT GTT CAA AGT ACT   351
Asn Ile Leu Lys Gln Leu Cys Glu Asp Trp Glu Val Val Pro Glu Pro Val Ala Arg Trp Cys Asn Val Gln Ser Thr

CAA GAT GAA TTT GAG GAA CTT ACA ATG TCT CAG AAA AAT GGT GGG AAT GTT CTT CAG ATG ATG TAT GAG AAA CCT GAA   529
Gln Asp Glu Phe Glu Glu Leu Thr Met Ser Gln Lys Asn Gly Gly Asn Val Leu Gln Met Met Tyr Glu Lys Pro Glu

CGA TGG TCT TTT ACC TTC CAA ACA TAT GCC TGT CTC AGT CGA ATA AGA GCT CAG CTT GCC TCT CTG AAT GGC AAG CTC   507
Arg Trp Ser Phe Thr Phe Gln Thr Tyr Ala Cys Leu Ser Arg Ile Arg Ala Gln Leu Ala Ser Leu Asn Gly Lys Leu

AAA GAT GCA GAG AAA CCT GTA TTA TTT TTT GAA CGA TCT GTG TAT AGT GAC AGG TAT ATT TTT GCA TCT AAT TTG TAT   585
Lys Asp Ala Glu Lys Pro Val Leu Phe Phe Glu Arg Ser Val Tyr Ser Asp Arg Tyr Ile Phe Ala Ser Asn Leu Tyr

GAA TCT GAA TGC ATG AAT GAG ACA GAG TGG ACA ATT TAT CAA GAC TGG CAT GAC TGG ATG AAT AAC CAA TTT GGC CAA   663
Glu Ser Glu Cys Met Asn Glu Thr Glu Trp Thr Ile Tyr Gln Asp Trp His Asp Trp Met Asn Asn Gln Phe Gly Gln

AGC CTT GAA TTG GAT GGA ATC ATT TAT CTT CAA GCC ACT CCA GAG ACA TGC TTA CAT AGA ATA TAT TTA CGG GGA AGA   741
Ser Leu Glu Leu Asp Gly Ile Ile Tyr Leu Gln Ala Thr Pro Glu Thr Cys Leu His Arg Ile Tyr Leu Arg Gly Arg

AAT GAA GAG CAA GGC ATT CCT CTT GAA TAT TTA GAG AAG CTT CAT TAT AAA CAT GAA AGC TGG CTC CTG CAT AGG ACA   819
Asn Glu Glu Gln Gly Ile Pro Leu Glu Tyr Leu Glu Lys Leu His Tyr Lys His Glu Ser Trp Leu Leu His Arg Thr

CTG AAA ACC AAC TTC GAT TAT CTT CAA GAG GTG CCT ATC TTA ACA CTG GAT GTT AAT GAA GAC TTT AAA GAC AAA TAT   897
Leu Lys Thr Asn Phe Asp Tyr Leu Gln Glu Val Pro Ile Leu Thr Leu Asp Val Asn Glu Asp Phe Lys Asp Lys Tyr

GAA AGT CTG GTT GAA AAG GTC AAA GAG TTT TTG AGT ACT TTG TGATCTTGCTGAAGACTACAGGCAGCCAAATGGTTCCAGATACTTCAG   987
Glu Ser Leu Val Glu Lys Val Lys Glu Phe Leu Ser Thr Leu

CTTTGTGTATCTTCGTAACTTCATATTAATATAAGTTTCTTTAGAAAACCCAAGTTTTTAATCGTTTTTGTTTTAAGGAAAAAAGATTTTTAAAATGAATCTTATG 1093
CAAAACTTTTTGATCAGTTTCTTTTCTTTTGTTTTTTTTTTAAAAAAGACATTTAAAGACAAAGACATTATTTCTCATAGCAGGAAATGTAGAGGTAGATGGTTCC 1199
AGTATCAGCATAGTGACTAAACTACATTATAAAAGATCCAGCTTCCTTCTGTCATTCCCCTCTTTTGTCTTCCTCAGCAGGTTGGCTTTTTTCCCTGGTGCCTCTC 1305
ACTTCGTTGGTGACCAGTTTCTTAAACTGAAAGCTTTAATGTTACATAGTAAATGGTAGTGTGTCCTGTGTAAATTAGTGTACCTATTAAAAGTTGCAAAGTGGAA 1411
TTAAAGGAATCCCTAGAATAAGGATTCTGAAGTTTTATTTTAAATTATTATCTTCTTAACAGTTTAGTCCCACCTCTTACTTCCTGCCTCAGTCTGCTTTCTCTAC 1517
TGTCTGGATTAATTAGGCAGCCTGCTATAAAGTTAAAGTCACACATTTCTATTTTGCAAACACTGTGATTACTCTTTGCTTTGTAGTTTGCTTTGCTTTGTAGGGT 1623
TCTGCTTTTAAGTTTTTCTCTTTTTCAGACAAATTACTGATAAAAATGATATTGCTCTATATGTAATATATCCTGAAAGCATTATTTTTTGTTGAATAGGA AATAA 1729
AATTAATGAAGACAGAGGCTAGAAAGCATCCATTAATTAATGAGACACACTTAACTACTTATCTCTAAACCATCTATGTGAATATTTGTAAAAATAATGAATGGAC 1835
TCATCTTAGTTCTGTATATAAATATATTTTCTTTCTAGTTTGTTTAGTTAAGGTGTGCAGTGTTTTTCCTGTGTATTAAACCTTTCCATTTTACGTTTTAGAAAAT 1941
TTTATGTATTTTAAAATAAGGGGAAGAGTCATTTTCACCTTTAAACTACTATTTTTCTTTCCAAGTCATTTTTGTTTTTGGTTTCTTATTCAAAGATGATAATTTA 2047
GTGGATTAACCAGTCCAGACGCACTGATCTTTGCAAAGGAGACTTAATTTCAAATCTGTAATTACCATACATAAACTGTCTCATTATACGTATGCATTTTTTTAGT 2153
TTGTTTTTGTTTGGTATAAATTAATTTGTTAATTAAATATTTCTTAAGTATAAACCTTATGAACTACAGTGGAGCTACACTCATTGAAATGTAATTTCAGTTCTAA 2259
AAAGATGTAATAATCATTTTAGAATTAAAATTTATTCTACTTTTA AATAAA TTATGAATATTAAAGGTGAAAATTGTATAAATTACTTTGATTCCATTTTAAGTGG 2365
AGACATATTTCAGTGATTTTTAGTAACCTTTAAAAATGTATAATGACTTTTAAAATTTGTAGAATTGAAAAGACGCT AATAAA AATTTATTATTTAAAAAAAAAA 2470
```

FIG. 1. cDNA and predicted amino acid sequence of dCyd kinase. Peptide sequences generated by tryptic digests are shown in boxes. A putative nucleotide-binding domain (positions 241–261; amino acids 28–34) and possible polyadenylylation signals are underlined.

for 1 hr at 65°C in 0.1× SSC/0.1% SDS and autoradiographed for 72–96 hr at −70°C.

Protein Expression in *Escherichia coli*. The pET-3d bacterial expression vector was kindly provided by F. W. Studier (25). The coding sequence of dCyd kinase cDNA was amplified by the polymerase chain reaction (PCR) technique using a 5′ sense primer containing an *Nco* I restriction site (ACAC-CATGGCCACCCCGCCCAAGAGAAGCT) and a 3′ reverse complement primer with a *Bam*HI site (CACGGATCCTCA-CAAAGTACTCAAAAACTCTTT). Amplified DNA was subcloned into pET-3d and transformed into *E. coli* strain BL21(DE3). The pET-3d vector alone was used as a control. Protein in bacterial lysate supernatants from centrifugation at 48,000 × *g* was analyzed by SDS/PAGE and by radiochemical assays for dCyd kinase activity (7).

Protein Expression in Mammalian Cells. The pDCK3B1A construct containing the 2.5-kb cDNA insert obtained from the CCRF-CEM library was cotransfected with the pSV2neo (26) vector into dCyd kinase-deficient murine L cells by standard techniques (27). L cells were also transfected with pSV2neo and an unrelated control insert (pAKE) obtained from the same library. Transfected cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum for 72 hr. The neomycin analog G418 was then added at 1 mg/ml. After 3 weeks, clusters of one to three colonies were expanded and assayed for dCyd kinase activity.

RESULTS

Isolation of dCyd Kinase cDNA. Of the tryptic peptides generated from ≈30 pmol of purified dCyd kinase, three could be sequenced. The amino acid sequences obtained were Trp-Cys-Asn-Val-Gln-Ser-Thr-Gln-Asp-Glu-Phe-Glu-Glu, Ile-Tyr-Leu-Arg, and Ala-Glu-Lys-Pro-Val-Leu-Phe-Phe-Glu. Based on 12 amino acids from the first peptide, a 35-base oligomer was synthesized using a combination of codon-usage bias and deoxyinosine substitution at degenerate positions (22) (TGCAACGTICAITCCACICAIGAT-GAITTCGAIGA). A 2.2-kb cDNA clone was isolated from a MOLT-4 cDNA λgt11 library on the basis of hybridization to this oligomer and was found to contain nucleotide sequence coding for all three peptides. Five additional cDNA clones ranging in size from 850 to 2460 base pairs (bp) were isolated from a CCRF-CEM cDNA expression library, and the full sequence of the largest clone, pDCK3B1A, was determined (Fig. 1).

The cDNA includes a 780-bp open reading frame encoding a protein with a predicted size of 30.5 kDa. There is a single potential N-linked glycosylation site at amino acid 148, and amino acids 28–34 (Gly-Asn-Ile-Ala-Ala-Gly-Lys) approximate the consensus nucleotide binding site Gly-Xaa-Xaa-Gly-Xaa-Gly-Lys (11). The cDNA clone also contains a 160-bp G+C-rich 5′ untranslated region and 1360 bp of A+T-rich 3′ noncoding sequence containing three potential polyadenylylation signals, the last located 18 bp from a poly(A) region (Fig. 1).

Bacterial Expression of dCyd Kinase. Isopropyl β-D-thiogalactopyranoside induction of T7 RNA polymerase activity in the pET-3d expression vector containing the cDNA insert resulted in the production of a protein of 30.5-kDa subunit molecular mass as seen on Coomassie-blue staining of SDS/polyacrylamide gels, whereas no distinct band was visible in this region for bacteria transformed with vector alone (Fig. 2). These data confirm that the dCyd kinase cDNA encodes a protein of the predicted subunit molecular mass.

Lysates of bacteria containing the pET-3d vector with or without insert were assayed for dCyd kinase activity. In the lysates from bacteria transformed with vector alone, dCyd kinase activity was <2-fold above background. In contrast, there was a 40-fold increase in dCyd kinase activity above background in lysates from bacteria transformed with the expression vector containing the dCyd kinase cDNA insert.

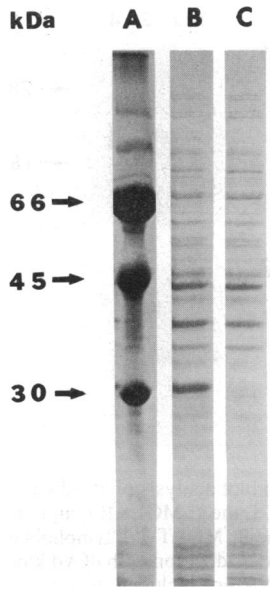

FIG. 2. SDS/PAGE of proteins from transformed BL21(DE3) cells. Lane A, Molecular weight standards; lane B, pET3d vector containing dCyd kinase coding region; lane C, pET3d vector alone.

Murine L-Cell Expression of dCyd Kinase Activity. Table 1 shows the results of assays for deoxynucleoside-phosphorylating ability in extracts from transfected murine L cells. Using dCyd, dAdo, and dGuo as substrates, the phosphorylating activities of lysates from cells transfected with the dCyd kinase cDNA construct were 427-, 14-, and 148-fold greater than control values, respectively. These results show that the protein encoded by this cDNA phosphorylates all three deoxyribonucleosides. To verify that the kinetics of this protein are similar to those of the purified dCyd kinase, the apparent K_m for dCyd was determined on a desalted cell extract and was found to be 1.0 μM. This value is very similar to apparent K_m values that we and others have previously reported for the enzyme (5–7).

Northern Blot Analysis. The dCyd kinase cDNA probe detected a single band of ≈2.8 kb for all lymphoid cell lines examined (Figs. 3 and 4). There was 5–10 times more dCyd kinase mRNA in MOLT-4 and Jurkat T lymphoblasts than in two B-lymphoblast lines (Fig. 3).

The levels of dCyd kinase mRNA were also determined in CCRF-CEM T lymphoblasts deficient in dCyd kinase activity. dCyd kinase mRNA levels in wild-type CCRF-CEM cells from our laboratory (Fig. 4, lane 4) and the HPRT-deficient parent CCRF-CEM line from Ullman's laboratory (Fig. 4,

Table 1. Expression of dCyd kinase activity in transfected murine L cells

Transfected plasmid	Velocity, pmol/(min·mg)		
	dCyd	dAdo	dGuo
pAKE (control)	0.2	9.9	5.1
pDCK3B1A	85.5	143.5	744.2

Murine L cells deficient in dCyd kinase activity were transfected with mammalian expression vectors containing pSV2neo and either the dCyd kinase coding sequence (pDCK3B1A) or an unrelated control sequence (pAKE) in a modified Okayama–Berg vector. Extracts from cells resistant to G418 were assayed for ability to phosphorylate dCyd (10 μM), dAdo (500 μM), or dGuo (500 μM) in a standard radiochemical assay.

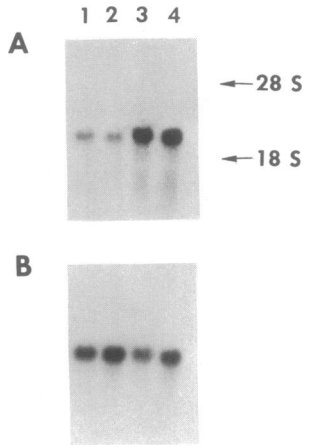

FIG. 3. Northern blot analysis of dCyd kinase mRNA levels in T and B lymphoblasts. Lane 1, MGL8 B lymphoblasts; lane 2, GM558 B lymphoblasts; lane 3, MOLT-4 T lymphoblasts; lane 4, Jurkat T lymphoblasts. (*A*) Hybridization with dCyd kinase cDNA. Positions of 28S and 18S rRNA are included as size markers. (*B*) The blot reprobed with β-actin cDNA.

lane 1) were comparable to levels in other T-lymphoblast cell lines (Fig. 3). In contrast, the level of dCyd kinase mRNA in the Ara-C-8D cells (Fig. 4, lane 2) was significantly decreased and that in ddCyd-resistant cells (Fig. 4, lane 3) was barely detectable. These data suggest that the functional dCyd kinase deficiency found in the resistant cell lines may be due to alterations in dCyd kinase mRNA expression.

DISCUSSION

dCyd kinase activity plays an integral role in the phosphorylation of three deoxyribonucleoside substrates and a broad spectrum of deoxyribonucleoside analogs used as antineoplastic and antiviral compounds. However, the low abundance and instability of this protein have hampered its purification and have led to disparate conclusions regarding its size and substrate specificity. We have definitively demonstrated that dCyd kinase activity resides in a protein of 30.5-kDa subunit molecular mass and that this protein is capable of phosphorylating dAdo and dGuo as well as dCyd. These data are in accord with previous reports on the purified enzyme by ourselves (7) and others (5). However, they conflict with recent data on affinity-purified enzyme from T

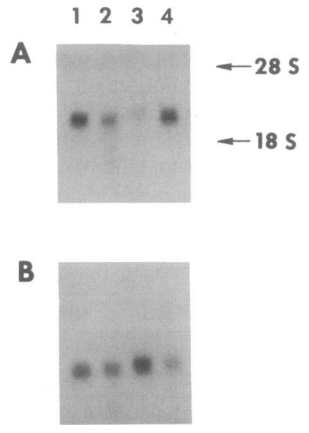

FIG. 4. Northern blot analysis of dCyd kinase mRNA levels in wild-type and mutant CCRF-CEM cell lines. Lane 1, HPRT-deficient cells; lane 2, araC-resistant cells; lane 3, ddCyd-resistant cells; lane 4, wild-type cells. (*A*) Hybridization with dCyd kinase cDNA. (*B*) The blot reprobed with β-actin cDNA.

lymphoblasts indicating that dCyd kinase is a 59.3-kDa monomer with proteolytic degradation products of 30 and 33 kDa (28). While it is possible that this protein represents a distinct deoxyribonucleoside kinase, it is clear that proteolysis does not account for the subunit molecular mass of dCyd kinase.

Based on kinetic studies using purified dCyd kinase preparations, there is a general consensus that a single enzyme catalyzes the phosphorylation of dCyd with a low K_m value (0.4–3.0 μM) and the phosphorylation of the purine deoxyribonucleosides dAdo and dGuo at significantly higher K_m values (120–890 μM for dAdo and 150–640 μM for dGuo) (4–6). We have demonstrated that the protein encoded by this cDNA has both the appropriate K_m for dCyd and the requisite multisubstrate specificity when expressed in murine L cells deficient in endogenous dCyd kinase activity. Given the possible existence of more than one kinase capable of phosphorylating dCyd in T lymphoblasts (29), this observation is of key importance in confirming that this protein is the dCyd kinase which has been extensively characterized. These properties also differentiate this protein from the 71-kDa product of the putative dCyd kinase cDNA reported by Huang *et al.* (16). However, those investigators have recently concluded that the protein encoded by their cDNA has strong structural similarity to the murine ERp72 protein (30) and probably represents the human counterpart to this endoplasmic reticulum protein (38).

An important structural feature of this protein is the presence of a probable nucleotide binding domain at amino acids 28–34. The consensus sequence Gly-Xaa-Xaa-Gly-Xaa-Gly-Lys has been reported in several ATP-binding enzymes including a variety of thymidine kinases, the *Lactobacillus* dCyd/dAdo kinase, and AMP deaminase (28). The sequence we have obtained indicates that the second glycine residue is replaced by an alanine in dCyd kinase. Since the clone that expressed dCyd kinase activity in the murine L cells contained the alanine residue at position 31, it is clear that this substitution does not prevent effective nucleotide binding. Analysis of the European Molecular Biology Laboratory (EMBL), GenBank, and National Biomedical Research Foundation databases (releases 24.0, 65.0, and 25.0, respectively) detected potentially significant sequence similarities to the Epstein–Barr virus thymidine kinase (EC 2.7.1.21), including a region of 22.6% amino acid identity in a 199-amino acid overlap beginning with the respective nucleotide binding domains and continuing through amino acid 218 of the dCyd kinase sequence. No other significant sequence similarities were detected in the analysis of a number of other kinase family members.

While the bacterial and L-cell expression data unequivocally point to the production of a catalytically active dCyd kinase, the altered mRNA levels in the two dCyd kinase-deficient CCRF-CEM lines provide direct evidence for the functional significance of this protein product. As demonstrated by Hershfield *et al.* (17), mutagenized CCRF-CEM cells selected for resistance to araC were less sensitive to growth inhibition by dAdo. Deficiencies of both dCyd kinase and adenosine kinase (EC 2.7.1.20) resulted in 100-fold decreased dAdo toxicity and the authors concluded that both enzymes participated in dAdo phosphorylation at concentrations <100 μM. Previous data had confirmed the resistance of dCyd kinase-deficient murine S49 cells to dGuo (31, 32). These observations led to the conclusion that a single dCyd kinase catalyzed the phosphorylation of all three deoxyribonucleosides in intact cells. Our data are consistent with this hypothesis and provide direct evidence for an alteration in the expression of dCyd kinase mRNA in both araC- and ddCyd-resistant cells.

The phosphorylation of dAdo and dGuo to their corresponding monophosphates by dCyd kinase is the rate-limiting

first step in the synthesis of dATP and dGTP, which serve as precursors of DNA synthesis and, at higher concentrations, as inhibitors of the enzyme ribonucleotide reductase (EC 1.17.4.1). Both dAdo and dGuo have been implicated in the pathogenesis of the immunodeficiency syndromes associated with adenosine deaminase deficiency (15, 33) and purine nucleoside phosphorylase deficiency (34), respectively. Cultured human T lymphoblasts and thymocytes are far more susceptible to dATP or dGTP accumulation in the presence of dAdo or dGuo than are B lymphoblasts or more mature lymphoid cells (35–37), and it has been hypothesized that dATP or dGTP accumulation is the mechanism of cytotoxicity for T-cell precursors in these disorders. This accumulation could occur as a consequence of increased dCyd kinase activity, decreased deoxyribonucleotide degradatory activity, or a combination of these factors (15). dCyd kinase activity in T-lymphoblast and thymocyte cell extracts is increased relative to other lymphoid cells (12–14), but whether this difference in activity is mediated at the level of gene expression or via modulation of enzyme activity by intracellular metabolites has not been clarified. Our data clearly indicate that the steady-state levels of mRNA for dCyd kinase are 5- to 10-fold higher in T lymphoblasts than in B lymphoblasts and strongly suggest that the differences in activity are due at least in part to increased expression of the gene or to increased stability of its mRNA in T lymphoblasts. This tissue-specific expression of dCyd kinase may partially explain the selective T-cell toxicity observed in adenosine deaminase and purine nucleoside phosphorylase deficiency.

We are grateful to Dr. Buddy Ullman for his generous gift of dCyd kinase-deficient cell lines and to Dr. Michael Clarke for the CCRF-CEM library. We also thank T. Ingland for her secretarial assistance. This work was supported by National Institutes of Health Grants 1RO1CA-34085, 5R29CA-46452, and K08-HL02061. D. Ginsburg is a Howard Hughes Medical Institute Investigator.

1. 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.
2. Durham, J. P. & Ives, D. H. (1970) *J. Biol. Chem.* **245,** 2276–2284.
3. Momparler, R. L. & Fischer, G. A. (1986) *J. Biol. Chem.* **243,** 4298–4304.
4. Datta, N. S., Shewach, D. S., Mitchell, B. S. & Fox, I. H. (1989) *J. Biol. Chem.* **264,** 9359–9364.
5. Bohman, C. & Eriksson, S. (1988) *Biochemistry* **27,** 4258–4265.
6. Sarup, J. C. & Fridland, A. (1987) *Biochemistry* **26,** 590–597.
7. Datta, N. S., Shewach, D. S., Hurley, M. C., Mitchell, B. S. & Fox, I. F. (1989) *Biochemistry* **28,** 114–123.
8. Dow, L. W., Bell, D. E., Poulakos, L. & Fridland, A. (1980) *Cancer Res.* **40,** 1405–1410.
9. Bhalla, K., Nayak, R. & Grant, S. (1984) *Cancer Res.* **44,** 5029–5037.
10. Ullman, B., Coons, T., Rockwell, S. & McCarton, K. (1988) *J. Biol. Chem.* **263,** 12391–12396.
11. Ikeda, S., Swenson, R. P. & Ives, D. H. (1988) *Biochemistry* **27,** 8648–8652.
12. Osborne, W. R. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4030–4034.
13. Staub, M., Spasokukotskaja, T., Taljanidisz, J., Sasvari-Szekely, M. & Antoni, F. (1983) *Immunol. Lett.* **6,** 137–142.
14. Kizaki, H., Habu, S., Ohsaka, F. & Sakurada, T. (1983) *Cell. Immunol.* **82,** 343–351.
15. Kredich, N. M. & Hershfield, M. S. (1989) in *Metabolic Basis of Inherited Disease*, eds. Scriver, C. R., Beauidet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), pp. 1045–1075.
16. Huang, S., Tomich, J. M., Wu, H., Jong, A. & Holcenberg, J. (1989) *J. Biol. Chem.* **264,** 14762–14768.
17. Hershfield, M. S., Fetter, J. E., Small, W. C., Bagnara, S., Williams, S. R., Ullman, B., Martin, D. W., Wasson, D. B. & Carson, D. A. (1982) *J. Biol. Chem.* **257,** 6380–6386.
18. Ginsburg, D., Handin, R. I., Bonthron, D. T., Donlon, T. A., Bruns, G. A. P., Latt, S. A. & Orkin, S. H. (1985) *Science* **228,** 1401–1406.
19. Clarke, M. F., Kukowska-Latallo, J. F., Westin, E., Smith, M. & Prochownik, E. V. (1988) *Mol. Cell. Biol.* **8,** 884–892.
20. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
21. Strohman, R. C., Moss, P. S., Micou-Eastwood, J., Spector, D., Przybyla, A. & Paterson, B. (1977) *Cell* **10,** 265–273.
22. Sambrook, J., Fritsch, E. F. & Maniatis, R. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
23. Gunning, P., Ponte, P., Okayama, H., Engel, J., Blau, H. & Kede, L. (1983) *Mol. Cell. Biol.* **3,** 787–795.
24. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
25. Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990) *Methods Enzymol.* **185,** 60–89.
26. Okayama, H. & Berg, P. (1983) *Mol. Cell. Biol.* **3,** 280–289.
27. Quackenbush, E., Clabby, M., Gottesdiener, K. M., Barbosa, J., Jones, N. H., Strominger, J. L., Speck, S. & Leiden, J. M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6526–6530.
28. Kim, M., Ikeda, S. & Ives, D. H. (1988) *Biochem. Biophys. Res. Commun.* **156,** 92–98.
29. Yamada, Y., Goto, H. & Ogasawara, N. (1985) *Int. J. Biochem.* **17,** 425–428.
30. Mazzarella, R. A., Srinivasan, M., Haigejorden, S. M. & Green, M. (1989) *J. Biol. Chem.* **265,** 1094–1101.
31. Ullman, B., Gudas, L. J., Cohen, A. & Martin, D. W., Jr. (1978) *Cell* **14,** 365–375.
32. Gudas, L. J., Ullman, B., Cohen, A. & Martin, D. W., Jr. (1978) *Cell* **14,** 531–538.
33. Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B. & Meuwissen, H. J. (1972) *Lancet* **ii,** 1067–1069.
34. Giblett, E. R., Amman, A. J., Wara, D. W., Sandman, R. & Diamond, L. K. (1975) *Lancet* **i,** 1010–1013.
35. Mitchell, B. S., Mejias, E., Daddona, P. E. & Kelley, W. N. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5011–5014.
36. Carson, D. A., Kaye, J. & Seegmiller, J. E. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5677–5681.
37. Carson, D. A., Kaye, J. & Seegmiller, J. E. (1978) *J. Immunol.* **121,** 1726–1731.
38. Huang, S. H., Tomich, J. M., Wu, H., Jong, A. & Holcenberg, J. (1991) *J. Biol. Chem.*, in press.