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Uridine Kinase: Altered Subunit Size or Enzyme Expression as a Function of Cell Type, Growth Stimulation, or Mutagenesis

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Using antibody prepared against pure uridine kinase from Ehrlich ascites cells, we have measured the expression of enzyme protein by the Western blot technique. Variations were observed in the M_r of the enzyme subunit for uridine kinase from different species: 32,000 (mouse Ehrlich ascites cells), 30,000 (normal human lymphocytes), 28,000 (mouse tissues), 27,500 (rat tissues). For different normal tissues from the same species, there was no significant variation in the subunit size. Transformed human and mouse cell lines, selected for a deficiency of uridine kinase activity in the presence of inhibitors activated by this enzyme, expressed two cross-reacting proteins, one with a normal (30,000) and one with a smaller (21,000) subunit molecular weight than was found in the parental cell line (human lymphoma), or only a smaller protein of M_r 25,000 (mouse lymphoma). Our results show that selection protocols using metabolite inhibitors do not always repress the expression of the enzyme but instead may lead to selection of those cells that have a mutation in the uridine kinase gene, resulting in the expression of an inactive enzyme. The expression of uridine kinase protein changes when cells are stimulated to divide. For both mouse fibroblasts and human lymphocytes, expression of uridine kinase protein as well as activity clearly increased after cells were stimulated to grow. In fibroblasts, increases are seen by 3 hr after stimulation, and plateau after 9 hr at a sevenfold increase. In lymphocytes, no change is seen until 12 hr after stimulation, and a plateau is not reached until 72 hr, with a total increase of ~50-fold. There has been considerable interest in the possibility of uridine kinase isozymes. Except for cells that have been mutagenized, the present results show that, as judged by subunit molecular weight, there appears to be only one enzyme form in normal and neoplastic cells or in cells in which uridine kinase activity is induced.

Key words: uridine kinase, subunit size of uridine kinase, induction, deficient mutants, enzyme expression of uridine kinase

Uridine kinase (ATP:uridine 5′-phosphotransferase; EC 2.7.1.48) catalyzes the phosphorylation of uridine and cytidine to form UMP and CMP. It has been shown

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for many cells and tissues that more UMP may be synthesized by this salvage route than by de novo synthesis [1–4]. Regulation of enzyme activity is balanced between the opposing effects of the substrate ATP and the feedback inhibitors UTP and CTP [5–7]. ATP stabilizes the enzyme in the active tetrameric form, whereas UTP and CTP dissociate the enzyme to the inactive monomer [5–7].

Additional modes of regulation are possible; it has generally been observed that, in cancerous, transformed, or neonatal cells, uridine kinase activity is considerably increased [1,2,8]. One hypothesis for this increased enzyme activity was that proliferating cells expressed a second isozyme, since multiple forms of uridine kinase were detected by several methods [9–24]. This interpretation appeared unlikely for Ehrlich ascites tumor cells, in which we have shown that only a single protein product is made [6] and that this protein is able, at 4°C, to associate into several slowly equilibrating polymers that readily separate by gel permeation chromatography or DEAE-cellulose chromatography [5–7].

With the recent purification of uridine kinase [6], it became possible, using antibodies raised against the pure mouse Ehrlich ascites enzyme, to examine the expression of enzyme protein in different tissues, mutated cell lines, and cells stimulated with growth factors. This approach also permits determination of whether altered expression of enzyme activity involves changes in the amount of enzyme protein or in the expression of additional enzyme species as judged by subunit molecular weight.

MATERIALS AND METHODS

Materials

Phytohemagglutinin (PHA) was obtained from Difco (Detroit, MI); [^{125}I] protein A was purchased from ICN (Irvine, CA); soybean trypsin inhibitor, aprotinin, leupeptin, pepstatin, chymostatin, phenylmethylsulfonylfluoride (PMSF), uridine, ATP, bovine serum albumin, deoxycholate, nonidet P-40, and sodium dodecyl sulfate (SDS) were obtained from Sigma Chemical Co. (St. Louis, MO). Triton X-100 was from Research International Corp. (Mount Prospect, IL). Whatman DE-81 filter discs were obtained from Whatman LTD (Hillsboro, OR). Bis-acrylamide, ammonium persulfute and polyacrylamide were purchased from BRL (Gathersburg, MD). Nitrocellulose sheets were obtained from Schleicher and Schuell (Keene, NH). Dulbecco's modified Eagle's medium (DMEM), Gibco RPMI-1640 defined culture medium, calf serum, and fetal calf serum (FCS) were obtained from the Cancer Research Center, University of North Carolina. Culture dishes were purchased from Fisher Scientific Co. (Raleigh, NC). The mutant human lymphoblastoid B-cell lines 6410/0, 6410/MP/DU, and 6410/MP/DU/AZUR were kindly supplied by Dr. N.K. Ahmed (St. Judes Hospital, Memphis TN). The mouse T-lymphoma cell lines S49, AU-11, AU-200-1, FU3-70G were gifts from Dr. B. Ullman (University of Kentucky). BALB/c-3T3 clone 31A was a gift from Dr. W.J. Pledger (Vanderbilt University).

Production of Antiuridine Kinase Serum

New Zealand white rabbits were primed with 200 μg of purified uridine kinase [6] mixed with one volume of Freund's complete adjuvant. The boosters were injected 13 and 42 days later using 100 μg and approximately 60 μg of purified uridine kinase,

respectively, in Freund's incomplete adjuvant. The immunizations were given subcutaneously on the necks and the backs of the rabbits. Sera were stored at −80°C.

Enzyme Assay

Uridine kinase activity was measured radiometrically using [5-^{3}H]uridine and ATP. The labeled product, UMP, was quantified by binding to Whatman DE-81 filter discs. A detailed description of the assay has been published elsewhere [6].

Tissue Survey

Various tissues from freshly sacrificed Sprague-Dawley rats and CF-1 mice were prepared by Dounce homogenization with an equal weight of buffer (10 mM KPO_4, pH 7.5, 1 μM pepstatin, 5 μM leupeptin, 5 μM chymostatin, 5 μl/ml aprotinin, and 100 μM PMSF). The supernatant was obtained by centrifugation in an Eppendorf microfuge for 15 min at 4°C.

Culture of Lymphoma Cells

Mouse T-cell lymphomas were cultured in a humidified incubator at 37°C with 5% CO_2. The cells were grown in suspension in DMEM containing 10% calf serum. This cell line was mutagenized with 1-methyl-3-nitro-1-nitrosoguanidine and selected for resistance to 6-azauridine and 5-fluorouracil [25]. Human lymphoblastoid B-cells were maintained similar to the mouse lymphomas, except in RPMI-1640 containing 10% fetal calf serum. These cell lines were mutagenized with 6-mercaptopurine and selected for resistance to 3-deazauridine and 6-azauridine as described by Ahmed et al [26].

Induction of Uridine Kinase in BALB/c-3T3 Fibroblasts

BALB/c-3T3 clone A31 cells were maintained in DMEM, 10% calf serum, at 37°C under a humidified atmosphere of 5% CO_2. Details of the culture procedures are described by Todaro and Green [27]. The cultures used in experiments were prepared by seeding into a 100-mm-diameter dish approximately 5×10^5 cells in 10 ml of medium. The medium was changed on day 3, and the cells were used 4 or 5 days later, when they had reached confluency; in this state, cells became starved in 2–3 days. Then, uridine kinase was induced in these quiescent cells by addition of fresh DMEM with 12% serum. Cultures were obtained at designated time points after serum activation. The cells were harvested by trypsinization and then washed twice with DMEM containing 10% serum and then once with DMEM containing 100 μg/ml of soybean trypsin inhibitor. The pelleted cells were then placed in digitonin-releasing buffer (20 mM HEPES, pH 7.5, 10 mM KPO_4, pH 7.5, 3 mM EDTA, 0.8 mg/ml digitonin, 0.25 mM sucrose, and 50 μl/ml aprotinin) for 5 min at 22°C [28]. The cell supernatant was obtained by centrifugation in a microfuge for 15 min. Three hundred microliters of digitonin-releasing buffer was used per culture dish.

Induction of Uridine Kinase in Human Lymphocytes

Fifty milliliters of human venous blood was collected, and 1,250 U of heparin was added. Red blood cells were removed by adherence to glass beads. Lymphocyte-containing plasma was then diluted 1:3 with RPMI-1640, layered on the bottom of Ficoll-Hypague, and centrifuged at 400g for 35 min. The layer containing lymphocytes was then removed and resuspended in RPMI-1640. The pellet obtained after

centrifugation at 160g for 15 min contained lymphocytes and polymorphonuclear granulocytes (PMN). The PMN were then removed by adherence to plastic culture dishes that had been coated with FCS. The final cell concentration was 10^6 cells/ml, of which 10 ml was placed per 100 mm dish. To induce uridine kinase, 2 μg of PHA was added per milliliter of cell culture. Human lymphocytes were grown in suspension, and at designated time points the cultures were terminated and cell supernatants were prepared as for the 3T3 cells above, except for the omission of trypsin and the use of 150 μl DRB per dish.

Western Blot

Proteins on a Laemmli [29] SDS-gel [10% acrylamide and 0.27% bisacrylamide (w/v)] were electrotransferred to nitrocellulose, which was processed according to Towbin et al [30], using 50 μl of antiserum in 10 ml 3% BSA Tris-saline buffer for each blot and 1 μCi [^{125}I] protein A per blot for detection of uridine kinase. The blot was then washed sequentially for 15 min in each of the following buffers: 1 M NaCl, 0.1% Nonidet P-40, 10 mM Tris (pH 7.2); 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 0.15 M NaCl, 50 mM Tris (pH 7.2); 0.05% Triton X-100, 0.15 M NaCl, 10 mM Tris (pH 7.2); and H_2O. The blot was then air dried and exposed to DuPont Cronex film for 1–2 days at −80°C.

To determine the amount of protein represented by bands on the autoradiogram, the film was scanned on a densitometer (Hoeffer Instruments model 65-300) to measure the absorbance. Peak areas were integrated automatically by an Apple IIe computer.

RESULTS

Mutant Cell Lines

Selection protocols using mutagens and nucleosides that become toxic after being phosphorylated by uridine kinase have produced several cell lines that are deficient in uridine kinase activity [25,26]. To study the expression of enzyme protein, we prepared rabbit antiserum raised against uridine kinase from mouse Ehrlich ascites cells, and this was effective at removing the enzyme activity from a partially purified sample of uridine kinase (Fig. 1), whereas preimmune serum was without effect. We used this antiserum in Western blots to examine the uridine kinase protein band, anticipating that the amount of enzyme protein would decrease in samples from cells deficient in uridine kinase activity.

What we observed were changes in the size of the uridine kinase subunit being expressed. As is shown in Figure 2, the enzyme from the mouse lymphoma parental line has a subunit M_r of 26,500, whereas the different mutant cell lines make a slightly smaller protein of 25,000.* The mutant lines analyzed in lanes 1 and 2 in Figure 2 are both derived from the AU-11 line shown in lane 4. By comparison, uridine kinase from Ehrlich ascites cells has a M_r of 32,000 (Fig. 2, lane 7). Since the amount of total protein in samples 1–4 (Fig. 2) was the same, it is apparent that the mutant cells also have lower quantities of uridine kinase protein.

*M_r values have been rounded off to the nearest 500 and in most cases represent the average of two or three determinations.

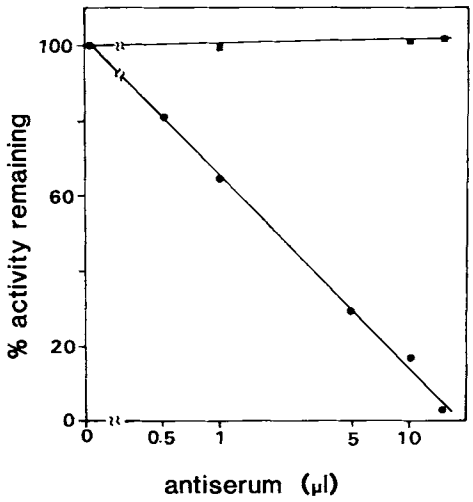

Fig. 1. Immunotitration of uridine kinase with antiserum. Enzyme samples (activity 20 nmole/min) were mixed with preimmune serum (■) or with antiserum against uridine kinase (●) and incubated for 30 min at 22°C followed by an additional 90 min at 4°C. To precipitate immune complexes, Protein A-Sepharose was added, and the mixture was further incubated for 60 min at 4°C. After centrifugation, enzyme activity was measured in the supernatant.

The results obtained with human lymphoma lines deficient in uridine kinase were somewhat different (Fig. 3). The parental line expresses an enzyme of M_r 30,000 (lane 7), whereas the mutant cells express two types of protein (lanes 8 and 9), at 30,000 and 21,000. The mutant cell line 6410/MP/DU/AZUR (lane 8) is derived from the mutant line 6410/MP/DU (lane 9). Data from the different lymphoma cell lines are summarized in Table I. The mouse lymphoma S-49 line has a normal level of uridine kinase activity. The mutagenesis and selection protocol for the AU 11 line leads to half the normal enzyme activity and the appearance of the altered subunit M_r. The two separate lines derived from AU 11 (AU-200-1 and FU3-70G) have the same subunit M_r and similar enzyme activity. For the human lymphomas, the parental 6410 line has very high enzyme activity. Mutagenesis and selection produced two lines that have greatly reduced uridine kinase activity (relative to the 6410 cell line), although these human lymphoma mutants have enzyme activity comparable to the mouse lymphoma mutants (Table I). However, the great reduction in uridine kinase activity for the human lymphoma mutants is accompanied by the appearance of a smaller subunit M_r species at 21,000 in addition to a reduced amount of the normal subunit M_r species at 30,000.

Figure 3 also illustrates that although different species produce a uridine kinase subunit of somewhat different size, each cell type (except the human lymphoma mutants) expresses only a single size of uridine kinase. Enzyme from different normal tissues of an animal has the same M_r, 27,500 for rat (Fig. 3, lanes 2 and 3) or 28,000 for mouse (data not shown). Table II summarizes all the subunit M_r values for uridine kinase from the various cells and tissues studied. Approximately normal specific activity is associated with enzyme having a size of 25,000–32,000; loss in enzyme activity is associated with subunits of 21,000.

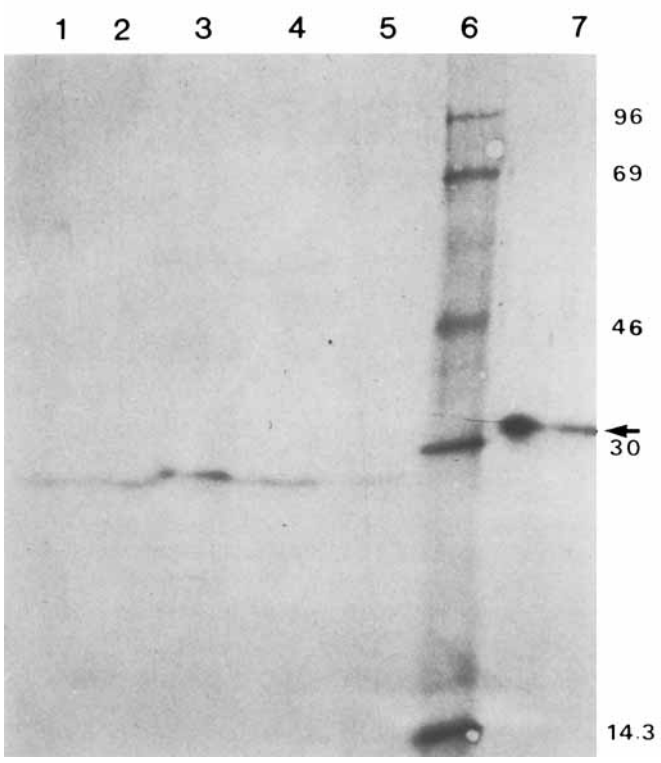

Fig. 2. Expression of uridine kinase protein in mouse lymphoma cells. Samples from different cell lines (with amount of protein) are **lane 1:** AU-200-1 (200 μg); **lane 2:** FU3-70G (200 μg); **lane 3:** S49 (wild-type; 200 μg); **lane 4:** AU 11 (200 μg); **lane 5:** AU 11 (100 μg); **lane 7:** Ehrlich ascites (2 μg): **Lane 6** contains molecular weight standards: myosin (200,000), phosphorylase (96,000), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and lysozyme (14,300). The arrow indicates the position of Ehrlich ascites uridine kinase.

Increased Uridine Kinase as a Function of Cell Replication

When quiescent cells are stimulated to begin replication, enzyme activity and enzyme protein both increase. In mouse fibroblasts activity is quite low in quiescent cells and increases sevenfold by 6 hr after cells are stimulated to enter G1 (Fig. 4A). Activity begins to level off at this point, increasing only modestly between 6 and 9 hr. In separate experiments, there was no further increase in activity between 9 and 24 hr (data not shown). As enzyme activity increases, the amount of cross-reacting protein also increases (Fig. 4A,B). For stimulated fibroblasts, enzyme activity increases more rapidly than enzyme protein, so that by 9 hr the specific activity of uridine kinase is twice that for quiescent cells. The pattern for enzyme increase is somewhat different in stimulated lymphocytes. Both enzyme activity and enzyme protein are almost undetectable in unstimulated cells (Fig. 5). Twelve hours after stimulation with PHA, both enzyme protein and uridine kinase activity have increased slightly (Fig. 5A). Thereafter, both activity and protein increase steadily, finally reaching a plateau at 72 hr. The overall increase in activity is about 50-fold (Fig. 5A), and the increase in protein over this time course is very comparable (Fig. 5B); there is no significant change in specific activity.

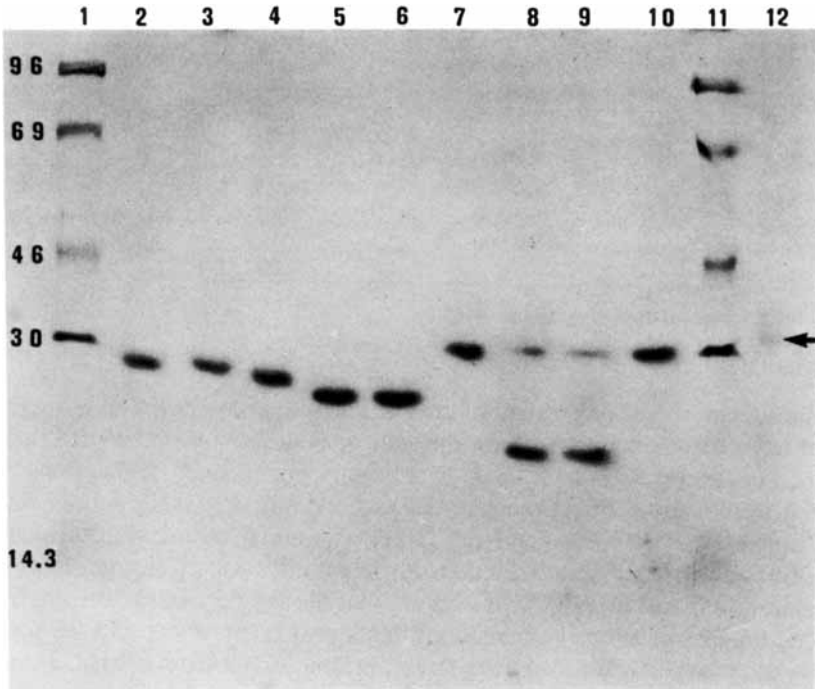

Fig. 3. Variations in subsunit size for uridine kinase from mouse, rat, and human tissues. Lanes contained the following protein samples with amount of protein. **Lanes 1** and **11:** M_r standards as listed in the legend to Figure 2; **lane 2:** rat liver (300 μg); **lane 3:** rat muscle (300 μg); **lane 4:** mouse lymphoma line S-49 (parental; 150 μg); **lane 5:** mouse lymphoma line AU-200-1 (150 μg); **lane 6:** mouse lymphoma line FU3-70G (150 μg); **lane 7:** human lymphoma line 6410 (parental; 60 μg); **lane 8:** human lymphoma line 6410/MP/DU/AZUR (80 μg); **lane 9:** human lymphoma line 6410/MP/DU (80 μg); **lane 10:** human lymphocytes (50 μg); **lane 12:** mouse Ehrlich ascites cells (5 μg). The arrow indicates the position of Ehrlich ascites uridine kinase.

TABLE I. Alteration in Both Enzyme Activity and Subunit Molecular Weight in Cell Lines Selected for a Deficiency in Uridine Kinase*

Cell line	Activity[a] (nmol/min/mg)	Uridine kinase-relative activity (%)	Subunit M_r
Mouse lymphoma			
S-49	8.2	100	26,500
AU 11	4.0	49	25,000
AU-200-1	2.2	27	25,000
FU3-70G	3.4	42	25,000
Human lymphoma			
6410	47	100	30,000
6410 MP/DU	1.2	2.1	30,000; 21,000
6410 MP/DU/AZUR	0.8	1.7	30,000; 21,000

*For the mouse lymphomas, the lines were derived S-49 → AU11 → (AU-200-1; FU3-70G). For the human lymphomas, the lines were derived 6410 → 6410 MP/DU → 6410 MP/DU/AZUR.
[a]Activity is expressed per milligram of protein in the supernatant of a cell homogenate.

TABLE II. Subunit Molecular Weights of Uridine Kinase

Source	Normal tissues	Wild-type tumor cells	Mutant tumor cells[a]
Mouse Ehrlich ascites		32,000	
Mouse tissues	28,000		
Mouse lymphoma		26,500	25,000
Rat tissues	27,500		
Human lymphocytes	30,000		
Human lymphoma		30,000	30,000; 21,000

[a]Selected for a deficiency in uridine kinase activity.

DISCUSSION

The subunit molecular weight of uridine kinase is constant for all normal tissues from rat or mouse but varies both for different species and for mutant cell lines from the same species. It is not clear if there is any significance to the variations in molecular weight for normal tissues or cells from mouse (28,000), rat (27,500), and human (30,000). It is interesting that, whereas normal mouse tissues all express uridine kinase with a subunit of 28,000, Ehrlich ascites cells from mouse produce a uridine kinase subunit of 32,000. It appears that the long-established Ehrlich ascites tumor cell line has developed a mutant uridine kinase that is about 15% larger.

We had previously estimated the size of uridine kinase from Ehrlich ascites cells at 31,000 [6], using 12% acrylamide gels and standard reference proteins. Here we have used 10% acrylamide gels and ^{14}C-methylated reference proteins and have consistently obtained a size of 32,000 for uridine kinase for the same pure enzyme. Although this illustrates that some variability in apparent molecular weight is observed as a function of methodology, and suggests caution in accepting as definitive any subunit M_r values reported here, the differences in subunit size are probably real since all values reported here were measured under identical conditions (eg, Fig. 3).

As is shown in Table I, there appears to be a difference of about 5,000 in the M_r for the subunit from two mouse tumors containing active uridine kinase: 32,000 (Ehrlich ascites cells) and 26,500 (mouse lymphoma S-49). Since the enzyme from the S-49 cells has not been purified or well characterized, it is not clear whether it lacks any of the regulatory features that we have shown to be important for controlling the activity of the Ehrlich ascites enzyme [5–7,31]. This size difference for uridine kinase suggests that the characterization of the smaller enzyme may provide a test for the hypothesis [32] that a correspondence exists between the subunit size of an enzyme and the total number of ligand binding functions for the enzyme.

Enzyme with a subunit size of 21,000 is associated with significant loss of enzyme activity and occurs only in selected, mutagenized cells. As was described in the Introduction, the possibility of uridine kinase isozymes has been of great interest; it may therefore be significant that only the defective mutant human lymphomas show more than one protein band (Fig. 3). Also, only a single protein band was observed in both fibroblasts (Fig. 4) and lymphocytes (Fig. 5) after enzyme activity was induced.

For the less active enzyme from mutant cells, we cannot be sure whether the antibody (prepared against uridine kinase from Ehrlich ascites cells) binds these with affinity comparable to the normal enzyme. Catalytically inactive enzyme may still bind to antibody and be detected by the immunoblot technique; the M_r 21,000 band

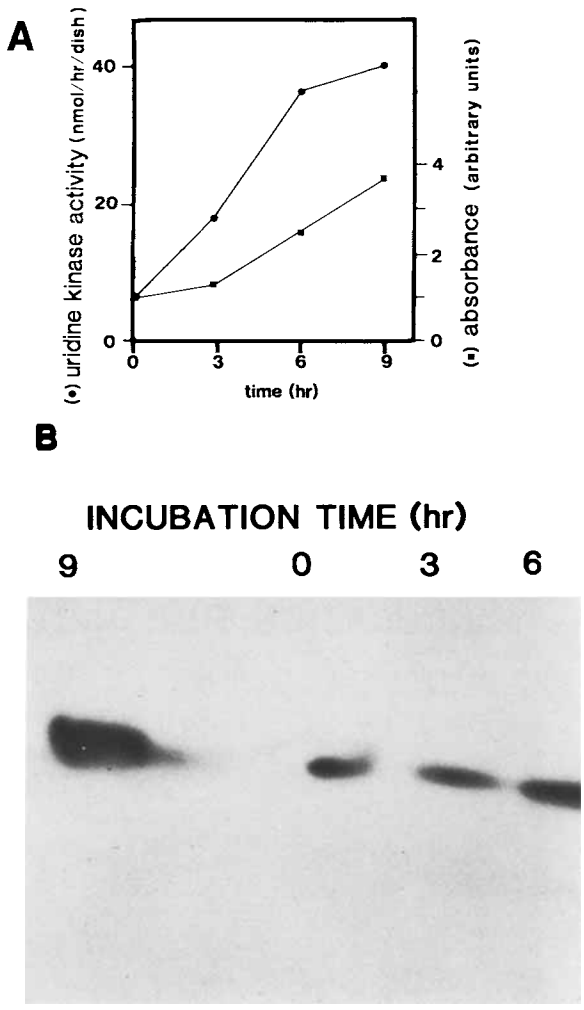

Fig. 4. Increased uridine kinase in mouse fibroblasts after cells are stimulated to growth with fresh serum. Cells were grown to confluence as described in Materials and Methods, and then fresh medium containing 12% serum was added at zero time. **A:** Activity of uridine kinase (●) is compared to the apparent amount of uridine kinase protein as measured by the absorbance (■) of bands on an autoradiogram of a Western blot (see B). **B:** Western blot with antibody to uridine kinase protein. Two microliters of cell supernatant was used in activity assays, and 80 μl was used for electrophoresis.

from the human lymphoma mutants (Fig. 3, lanes 8 and 9) is assumed to be an example of this. The most reasonable interpretation is that the M_r 30,000 band contains the minimal remaining activity of about 2% (Table I). The amount of protein in the M_r 30,000 band appears to be somewhat greater than 2% of the total uridine kinase protein detectable in lanes 8 and 9 in Figure 3. Therefore, the immunoblotting probe may not be comparably linear for the mutant enzymes. An alternative explanation is that the single mutation induced in these sublines leads to expression of an enzyme that 1) has lower catalytic activity but the same M_r as the wild-type enzyme and 2) is more susceptible to proteolysis, leading to significant conversion to the (presumably) catalytically inactive M_r 21,000 protein. Therefore, the percent remain-

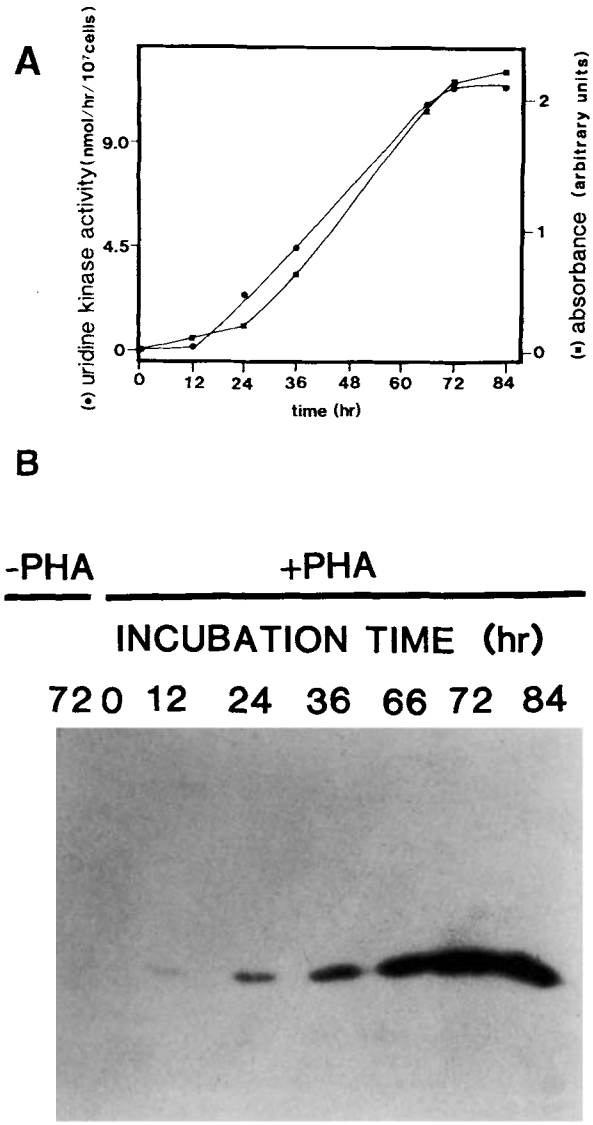

Fig. 5. Increased uridine kinase in human lymphocytes after cells are stimulated to growth with PHA. Cells were grown as described in Materials and Methods. PHA was added to cell cultures at zero time. **A:** The activity of uridine kinase (●) is compared to the apparent amount of uridine kinase protein as measured by the absorbance (■) of bands on an autoradiogram of a Western blot (see B). **B:** Western blot with antibody to uridine kinase. Two microliters of cell supernatant was used in activity assays, and 80 μl was used for electrophoresis.

ing uridine kinase activity need not correspond directly to the proportion of M_r 30,000 protein.

The mutant cell lines were made deficient in uridine kinase activity by a selection protocol in which cells were exposed to mutagens and inhibitors requiring uridine kinase for activation. Such a selection strategy should lead to the survival of cells having a mutation in the uridine kinase gene causing decreased transcription or

production of a less active enzyme. Our results show that the mutant human lymphoma cells make amounts of a defective protein that are equal to or even greater than the amount of enzyme made by the parental cells (Fig. 3), whereas the mutant mouse lymphoma cells appear to express less protein than the parental cells (Fig. 2).

Point mutations are usually produced by the mutagens 6-mercaptopurine [33] and 1-methyl-3-nitro-1-nitrosoguanidine [34]. There are several likely mechanisms by which a point mutation could result in a smaller protein: 1) later initiation of transcription or translation, 2) earlier termination of transcription or translation, or 3) greater sensitivity to proteolytic cleavage. These mechanisms all lead to a truncated protein. A point mutation that changes a splicing site as RNA is processed could also lead to a smaller final product. Some normal genes code for RNAs that undergo different splicing patterns to produce proteins of altered size. Thus alteration in splicing leads to a minor ovomucoid protein differing by only two amino acids [35] and to two types of myosin light chain that vary more significantly at their amino terminus [36].

As a rate-limiting enzyme that provides nucleotide precursors for the continuous synthesis of RNA, uridine kinase may well be considered a housekeeping enzyme and therefore be expected to be expressed constitutively. Alternatively, regulation of expression of this enzyme may be coupled to the periodic synthesis of DNA. Support for the induction of uridine kinase came from studies reporting increased enzyme activity in homogenates of cells or tissues after some form of stimulation: 1) a twofold increase in rat liver following treatment with alloxan [37]; 2) a three to fivefold increase in rat liver after administration of 5-azacytidine [38]; 3) a three to fivefold increase in mouse fibroblasts stimulated with serum, or platelet derived growth factor, or platelet-poor plasma [39]; 4) an increase of three to fourfold in rat or pig lymphocytes stimulated with PHA [17,40]; and 5) an increase of ~20–40-fold after stimulation of human lymphocytes with PHA [41–43]. However, after treatment with 5-azacytidine, uridine kinase activity was decreased in mouse EL-4 leukemia cells [17] and in rat thymus [44].

Since uridine kinase is regulated by several nucleoside triphosphates [5–7], it was possible that increases in nucleotide pools, consequent to stimulation by mitogens, contributed to the altered uridine kinase activity. Our results (Fig. 5) show that enzyme protein increases in a fashion corresponding with increases in enzyme activity for human lymphocytes. With mouse fibroblasts (Fig. 4), there is a modest increase in the amount of enzyme protein after 3 hr, and this is reasonable for the time required to detect an increased rate of transcription and translation. This time course also explains why an earlier study by Rozengurt et al [45] failed to detect any change in uridine kinase activity after stimulation of Balb3T3 cells with epidermal growth factor. These authors made only one measurement, at 1 hr after exposing their cells to growth factor. After induction of the fibroblasts, enzyme activity increases much more rapidly than enzyme protein, suggesting that additional mechanisms may alter the intrinsic activity of uridine kinase existing at 0 hr. For lymphocytes, the time course is slower, but cells do not go through a cell cycle before expression of uridine kinase is increased. Mitosis normally begins about 3 days after stimulation with PHA [46], by which time maximum expression of uridine kinase has been achieved (Fig. 5).

It is interesting to compare the induction of uridine kinase with that of deoxythymidine kinase. The latter enzyme supplies nucleotides only for DNA synthesis. Its

activity is very low in nondividing cells and then increases dramatically (> 100-fold) as cells enter S phase [47]. Thus, although the expression of both enzymes is increased by the same mitogenic factors, the time course for their induction is significantly different.

ACKNOWLEDGMENTS

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