Purification and Properties of Estrogen-responsive Cytoplasmic Thymidine Kinase from Human Breast Cancer

Diane A. Bronzert, Marie E. Monaco, Lawrence Pinkus, Susan Aitken, and Marc E. Lippman

ABSTRACT

The effect of 17β-estradiol on cytoplasmic thymidine kinase activity was studied in MCF-7, a human breast cancer cell line in culture which responds to estrogens with an increase in the rate of growth. Levels of 17β-estradiol which maximally stimulate [3H]thymidine incorporation into DNA also maximally stimulate thymidine kinase activity. The Vₘₐₓ for thymidine increased while the Kₘ was not affected by estrogen stimulation when performed on nonpurified enzyme. Tamoxifen, an antiestrogen, decreased the specific activity of the enzyme.

To further study its hormonal regulation, cytoplasmic thymidine kinase was purified greater than 2000-fold by affinity column chromatography. The purified preparation migrated in one band to a pI of 8.5 on an isoelectric focusing gel. The purified thymidine kinase was further characterized by examining its molecular weight, pH optimum, heat stability, utilization of phosphate donors, inhibition by nucleotides, and the effect of pyrimidine nucleoside analogs.

INTRODUCTION

The effects of estrogens on growth and proliferation have been described for a variety of target tissues (3), and many mammary tumors have been found to require estrogen for growth (22). We have reported previously that MCF-7, a human breast cancer cell line in continuous tissue culture (6) which possesses specific estrogen receptors (2), responds to estrogens with an increase in overall macromolecular synthesis and growth (11). The mechanism by which estrogen induces this increase in growth and proliferation is not known. We have shown that thymidine incorporation is enhanced 2- to 5-fold by physiological concentrations of 17β-estradiol (11).

In other cell systems, an increase in DNA synthesis has been paralleled by an increase in the activity of cytoplasmic thymidine kinase (EC 2.7.1.21), an enzyme of the salvage pathway of deoxynucleotide biosynthesis which converts thymidine to thymidylate. When we looked at thymidine kinase activity following estrogen treatment, the specific activity increased 1.5- to 4-fold. To further study this hormonal effect, we purified and characterized cytoplasmic thymidine kinase from the MCF-7 cell line.

MATERIALS AND METHODS

Materials. CH-Sepharose 4B was purchased from Pharmacia Fine Chemicals (Piscataway, N. J.). p-Aminophenythymidine 3'-phosphate was purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide from Eastman Organic Chemicals Division (Rochester, N. Y.). The nucleotides and nucleosides were purchased from the Sigma Chemical Company (St. Louis, Mo.) or Boehringer Mannheim. [3H]Thymidine (46 Ci/mmol) was obtained from Amersham/Searle Corp. (Arlington Heights, Ill.). DE81 chromatography filter papers were purchased from Whatman, Inc. (Clifton, N. J.).

Cell Line. MCF-7 is a human breast cancer cell line derived from a malignant pleural effusion of a postmenopausal woman with metastatic breast cancer. Starter cultures were generously provided by Marvin Rich of the Michigan Cancer Foundation. This cell line has been shown to be free of Mycoplasma contamination (Flow Laboratories, Rockville, Md.). The human and mammary nature of these cells has been extensively documented (2, 6, 11, 12, 20).

Cells were grown in monolayer culture in IMEM plus 2× glutamine, penicillin, and streptomycin (NIH Media Unit) (18), supplemented with 10% fetal calf serum (North American Biologicals, Inc., Miami, Fla.), in a humidified incubator at 37° with 5% CO₂ and 95% air.

Stimulation Experiments. In experiments involving estrogen stimulation of thymidine kinase activity, cells were replated into 100-mm sterile plastic Petri dishes. When the cells had attached to the surface of the dish, the medium was changed to IMEM without serum. The medium was exchanged for fresh serum-free IMEM 8 hr later. Following an additional 24 hr serum-free incubation, hormones were added. At designated times after hormone addition, cells were either (a) labeled with [3H]thymidine for 1 hr, and incorporation of tritium into acid-precipitable material was measured as described previously (12), or (b) harvested for measurement of thymidine kinase activity. For thymidine kinase assays, cells were washed with cold Dulbecco’s phosphate-buffered saline (pH 7.4) without calcium or magnesium and harvested by scraping with a rubber policeman. Cells were collected at high speed in a Clay-Adams serofuge. The cell pellet was resuspended in 0.05 M Tris-HCl buffer (pH 8.0) containing 0.25 mM sucrose. The cells were disrupted with 30 strokes of a tight-fitting Dounce homogenizer, and the homogenate was centrifuged for 10 min at 105,000 × g at 4° in a Beckman LS-65 ultracentrifuge. The resulting supematant was used for assay of thymidine kinase activity.

Measurement of Binding of [3H]Estradiol to Receptor. Methods used for assessing specific binding of tritiated estradiol to intact MCF-7 cells have been described previously (10). Assay of Enzyme Activity. Deoxynucleoside kinase activity was measured using a modification of the method of Breitman (1). The assay mixture contained [3H]thymidine (20 to 50 μM),...
0.005 M ATP, 0.005 M MgCl₂, 0.25 M Tris-HCl buffer (pH 7.8), and the thymidine kinase preparation in a final volume of 0.1 ml. In reactions using purified thymidine kinase preparations, 0.1% bovine serum albumin was added to the reaction mixture. The assay mixture was incubated at 37°C for various time intervals up to 30 min, and the reaction was stopped by pipetting 25 µl of the reaction mixture on a Whatman DE81 filter disc or by boiling for 5 min prior to pipetting the mixture on a filter disc. The discs were immediately dropped into 1 mM ammonium formate for 3 washes and then washed with ethanol. The disc was dried and then inserted into a vial containing Aquasol (New England Nuclear), and the radioactivity was determined in a Packard Tri-Carb Model 3490 scintillation counter. The reaction was shown to be linear with time. One unit of thymidine kinase activity is defined as the amount of enzyme which can convert 1 nmol of thymidine to TMP per min under the assay conditions described. Protein was determined by the method of Lowry et al. (13).

**Purification.** The preparation of the thymidine kinase from MCF-7 cells was done by a modification of the method used by Lee and Cheng (9). MCF-7 cells were grown to approximately 80% confluency in forty 450-sq cm roller bottles in the media described previously. Approximately 2.5 × 10^10 cells were harvested from the roller bottles by washing once with Dulbecco’s phosphate-buffered saline followed by 0.02% EDTA in Puck’s Saline A (NIH Media Unit). Cells were spun at 800 × g for 5 min in an IEC Model PRJ centrifuge to remove the buffer and then stored at −70°C until used. The frozen MCF-7 cell pellets were defrosted and resuspended in 0.01 M Tris-HCl (pH 7.5), 10% glycerol, 2 mM DTT, and 0.5 mM EDTA. The cells were homogenized on ice with 30 strokes of a Potter-Elvehjem homogenizer. This crude cell homogenate was centrifuged at 105,000 × g for 1 hr in a Beckman L5-65 ultracentrifuge. The supernatant was then subjected to a streptomyacin sulfate fractionation by adding 20% streptomyacin sulfate dropwise to obtain a final concentration of 2% (v/v). After being stirred for 15 min, the solution was centrifuged at 12,000 rpm for 20 min in a Beckman J-21 centrifuge, and the supernatant was frozen at −70°C or applied directly to the affinity column. All procedures were performed at 4°C.

The mitochondrial thymidine kinase was isolated by a modification of the procedure used by Kit et al. (4). A fresh MCF-7 cell pellet was washed with 0.01 M Tris-HCl buffer (pH 7.4), 0.001 M EDTA, 0.01 M NaCl, and 0.25 M sucrose and then resuspended and allowed to swell for 10 min at 4°C. The cells were then homogenized with 30 strokes of a Potter-Elvehjem homogenizer and centrifuged twice at 1500 × g for 10 min in an IEC Model PRJ centrifuge to remove the nuclei. A mitochondrial pellet was prepared by centrifuging the supernatant at 12,000 × g for 10 min and washing 3 times. The resulting pellet was resuspended in 0.01 M Tris (pH 8), 0.15 M KCl, 0.003 M 2-mercaptoethanol, and 0.5% Nonidet P-40 and sonicated. The sonicate was centrifuged at 105,000 × g for 60 min, and the supernatant was frozen at −70°C for further use. All procedures were performed at 4°C.

**Preparation of Affinity Column.** The affinity column matrix was prepared using the procedure of Kowal and Markus (7). L-Aminophenylthymidine 3'-phosphate was synthesized by reducing L-nitrophenylthymidine 3'-phosphate under 2 atmospheres of P_H₂ in the presence of 10% palladium on carbon. The unreacted compound was removed by passing the filtered product through a Dowex 50-H⁺ column and eluting with water. Fifteen mg of L-aminophenylthymidine 3'-phosphate were linked onto 6 ml of CH-Sepharose 4B in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. Any unreacted carboxyl groups on the matrix were then blocked with ethanolaamine. An estimated 12 mg of thymidine derivative were bound to the CH-Sepharose 4B.

**Purification of Thymidine Kinase from Human Breast Cancer**

**Effect of Estrogen on Thymidine Incorporation, Thymidine Kinase Activity, and Binding of Estradiol to Estradiol Receptor.** Chart 1 illustrates the effects of increasing concentrations...
MgCl₂ were kept at a fixed concentration while the thymidine concentration was varied from 0.6 to 50 μM.

**Effect of Tamoxifen on Thymidine Kinase Activity.** We have previously shown that the antiestrogen, tamoxifen [ICI 46474; (trans-1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenyl-1-but-1-ene citrate)], causes a decrease in incorporation of [³H]thymidine into acid-precipitable material (11, 12). This inhibition is prevented by simultaneous addition of estrogen and reversed by the subsequent addition of estradiol for up to 48 hr, suggesting some common mechanism for the action of the 2 compounds (12). Furthermore, human cell lines lacking estrogen receptor are not inhibited by tamoxifen (11). We examined the effect of tamoxifen on thymidine kinase activity and found a decrease in activity as seen in Table 1.

<table>
<thead>
<tr>
<th>Addition</th>
<th>No. of experiments</th>
<th>% of control²</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol (10⁻⁸ M)</td>
<td>16</td>
<td>196 ± 3³</td>
</tr>
<tr>
<td>Tamoxifen (2 x 10⁻⁶ M)</td>
<td>10</td>
<td>57 ± 2</td>
</tr>
<tr>
<td>Tamoxifen + 17β-estradiol</td>
<td>10</td>
<td>172 ± 7</td>
</tr>
</tbody>
</table>

³ Activity was measured 24 hr after estradiol or tamoxifen addition.

**Chart 1.** Effect of 17β-estradiol on thymidine incorporation and thymidine kinase activity, and specific binding of 17β-estradiol to receptor in MCF-7 cells. Thymidine incorporation and thymidine kinase activity were measured 24 hr after estradiol addition.

of 17β-estradiol on thymidine kinase activity and thymidine incorporation in MCF-7 cells. Binding of [³H]17β-estradiol to specific cytosol receptors is also shown. A greater than 2-fold increase is seen in thymidine kinase activity with estradiol concentrations in excess of 10⁻⁹ M. The increase in incorporation of [³H]thymidine follows an estradiol dose-response curve that is nearly colinear with that observed for thymidine kinase activity. The binding curve of 17β-estradiol to receptor lies significantly to the right of these 2 curves. We have previously observed this dose-response relationship between binding and thymidine incorporation whether binding is measured on cytosols at 0° (16) or in intact cells at 37° (12). This discrepancy could be accounted for if only a small proportion of cytoplasmic receptor sites need to be occupied in order to maximally induce thymidine kinase activity. This point is discussed later. The estradiol-induced increase in thymidine kinase activity is apparent in cells plated over a 20-fold range of density (2 x 10⁵ to 4 x 10⁶ cells/dish), with a slightly greater response at lower cell densities.

**Time Course.** If an increase in thymidine kinase activity were involved in the mechanism by which estrogens induce an increase in growth and proliferation, one might reason that the increase in thymidine kinase activity would precede or coincide with the increase observed in incorporation of ³H precursors into DNA. Chart 2 illustrates that this is indeed the result when thymidine kinase activity and thymidine incorporation are measured as a function of time of incubation with 10⁻⁸ M 17β-estradiol. Stimulation of thymidine kinase activity paralleled increased thymidine incorporation. In this experiment, there appeared to be a slight increase at 8 hr before any effect of estradiol on thymidine incorporation was observed; however, this effect at early times was quite variable.

**Kinetics of Estrogen Stimulation of Thymidine Kinase.** To further study how estrogen stimulates thymidine kinase activity, kinetic studies were performed on the cytosol extracts from the MCF-7 cells. The cells were grown under the conditions described in "Stimulation Experiments" and then stimulated with 10⁻⁸ M 17β-estradiol for 24 hr. A 105,000 x g cytosol was made from both unstimulated and stimulated cells and used in the substrate saturation experiment shown in Chart 3. ATP and

**Chart 2.** Time course of the effect of 17β-estradiol on thymidine incorporation and thymidine kinase activity in MCF-7 cells.

**Chart 3.** Lineweaver-Burk plots of thymidine kinase activity from 17β-estradiol-stimulated and control MCF-7 cytosols. The enzyme assays were carried out as described in "Materials and Methods" with varying amounts of thymidine (1/35).

**Table 1**

| Effect of 17β-estradiol and tamoxifen on thymidine kinase activity² in MCF-7 cells |
|---------------------------------|-------------------|-------------------|
| Addition                        | No. of experiments | % of control²     |
| 17β-Estradiol (10⁻⁸ M)          | 16                 | 196 ± 3³          |
| Tamoxifen (2 x 10⁻⁶ M)          | 10                 | 57 ± 2            |
| Tamoxifen + 17β-estradiol       | 10                 | 172 ± 7           |

³ Activity was measured 24 hr after estradiol or tamoxifen addition.

² Control value was 228 ± 8 pmol/mg/min.

³ Mean ± S.E.
Furthermore, no inhibition was seen when the antiestrogen and estradiol were added simultaneously.

**Enzyme Purification by Affinity Chromatography.** Thymidine kinase will preferentially bind to the immobilized thymidine analog on CH-Sepharose 4B, while the other cytosol proteins essentially come through in the void volume. The p-amino-phenylthymidine 3'-phosphate affinity column was washed with 0.01 M Tris (pH 7.5), 2 mM DTT, and 10% glycerol and then transferred to a tube. The streptomycin sulfate fraction was added, and the tube was gently shaken at 4°C on an Ames aliquot mixer for 30 min. The slurry was transferred to a 1.5- x 10-cm column and eluted in 4-ml fractions. Chart 4 shows the elution conditions used for the column. The column was first washed with 0.2 M Tris, 2 mM DTT, and 10% glycerol to remove any nonspecifically bound proteins. Other investigators have shown that any contaminating deoxythymidine phosphorylase is eluted with this high-salt fraction (7, 8). The thymidine kinase was eluted in the above buffer with the addition of 500 μM thymidine. Thymidine competes with the immobilized ligand for thymidine kinase, and approximately 50% of the thymidine kinase activity was recovered. When the salt and thymidine concentration of the eluting buffer were increased and the column was washed again, the recovery was improved with the release of another small peak of thymidine kinase. This peak had the same Rf on a 5% nondissociating polyacrylamide gel as did the first peak of thymidine kinase. Thus, the low recovery from the affinity column may be due to trapping or irreversible absorption to the gel. The fractions containing thymidine kinase were combined and dialyzed in 0.01 M Tris (pH 7.5), 2 mM DTT, and 10% glycerol and reapplied to the second affinity column for further purification. As seen in Table 2, the recovery from this column was only 33%, which may be due to the small amount of protein applied and the lability of the enzyme as it reached homogeneity. The overall purification was greater than 2000-fold.

**Electrophoresis of Cytoplasmic Thymidine Kinase.** To demonstrate homogeneity and to further characterize the cytosol thymidine kinase, the purified preparation was applied to a 5% nondenaturing polyacrylamide gel. As shown in Chart 5, a single protein band with an electrophoretic mobility of 0.11 can be seen. An identical gel was sliced, and an enzyme assay

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**Table 2**

<table>
<thead>
<tr>
<th>Step</th>
<th>Fraction</th>
<th>Vol (ml)</th>
<th>Protein (mg/ml)</th>
<th>Activity (units/ml)</th>
<th>Specific activity (units/mg)</th>
<th>Purification</th>
<th>Total (units)</th>
<th>% of yield</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>Homogenate</td>
<td>100</td>
<td>25.34</td>
<td>33.16</td>
<td>1.30</td>
<td></td>
<td></td>
<td>3316</td>
</tr>
<tr>
<td>2</td>
<td>Cytosol</td>
<td>80</td>
<td>18.17</td>
<td>31.62</td>
<td>1.74</td>
<td>1.3</td>
<td>2526</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>Streptomycin sulfate precipitate</td>
<td>80</td>
<td>13.25</td>
<td>26.08</td>
<td>1.97</td>
<td>1.5</td>
<td>2086</td>
<td>63</td>
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<td>Affinity Column 1</td>
<td>50</td>
<td>0.088</td>
<td>21.12</td>
<td>240.0</td>
<td>183.0</td>
<td>1056</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>Affinity Column 2</td>
<td>18</td>
<td>0.008</td>
<td>21.10</td>
<td>2638.00</td>
<td>2026.0</td>
<td>379</td>
<td>11</td>
</tr>
</tbody>
</table>

Protein values in Steps 4 and 5 obtained by precipitating sample and standards with an equal volume of 20% trichloroacetic acid and then running the Lowry assay.

A unit is defined as the amount of enzyme which can convert 1 nmol of thymidine to TMP per min.

Specific activity is obtained by dividing activity (Column 5) by protein (Column 4).

Purification is obtained by dividing the specific activity for each step by the specific activity of the homogenate.

Total unit value is obtained by multiplying the activity (Column 5) by the volume (Column 3).

Percentage of yield is found by dividing the total units (Column 8) of each step by the total units of the homogenate.
was performed as described in "Materials and Methods." A single peak of thymidine kinase activity was found with the same migration pattern as the protein band.

Isoelectric focusing was also performed on the purified thymidine kinase as described in "Materials and Methods." The thymidine kinase migrated in one band to a pl of 8.5 as shown in Chart 6.

**Electrophoresis of Mitochondrial Thymidine Kinase.** Mitochondria were purified from MCF-7 cells as described previously to check for the existence of a second thymidine kinase in these cells and to eliminate this isozyme as a source of contamination. The mitochondrial sonicate was found to have a thymidine kinase specific activity of 0.06 unit/mg/min. This represents 0.2% of the total activity found in the preparation. When this sonicate was applied to an isoelectric focusing polyacrylamide gel, a large peak of activity migrated to pH 5.0, and a small one was found at pH 8.5. The pl 8.5 peak may represent contamination from the cytosol (data not shown).

**Properties of Thymidine Kinase.** To further characterize the purified thymidine kinase, several molecular properties of the enzyme were investigated. The molecular weight of cytoplasmic thymidine kinase was estimated by applying the purified enzyme to a 5% SDS-polyacrylamide gel in the presence of β-mercaptoethanol. An estimated molecular weight of 177,000 was obtained using thyroglobulin, ferritin, phosphorylase b, and albumin as markers (Chart 7).

The effect of pH on thymidine kinase activity was studied by varying the pH of the reaction buffer with 0.1 M Tris-maleate (pH 5.0 to 6.8) or 0.1 M Tris-HCl (pH 7.0 to 9.7). The purified enzyme was found to be maximally active at pH 8.0 as stated in Chart 8.

Temperature inactivation of the cytoplasmic thymidine kinase was shown by preincubating the purified enzyme for 5 min at graded temperatures of 0–70°C. The enzyme was immediately cooled in ice and then assayed as described previously in "Materials and Methods." The enzyme exhibited a sharp decrease in activity above 35°C with a loss of 45% at 45°C.

Kinetic studies were also performed on the purified enzyme, but, due to the difficulty in obtaining an accurate protein value, we were unable to calculate the V_max. The K_m for the purified thymidine kinase was 5 μM, which correlates well with the cytosol values previously given.

**Utilization of Phosphate Donors by Thymidine Kinase.** Investigators have previously compared the ability of thymidine kinase from human fetal and adult liver to utilize various phosphate donors. Maximal activity was obtained for fetal liver when ATP and dATP were used as substrates, while adult liver could also utilize CTP, UTP, and GTP (21). When we examined the ability of purified cytoplasmic thymidine kinase from MCF-7 cells to utilize various triphosphates, dATP gave 89% of the activity of ATP. The enzyme was unable to use CTP, UTP, GTP, or their corresponding ribonucleotides (Chart 9).

**Inhibition of Thymidine Kinase Activity by Nucleotides.** To study the effect of various nucleotides on purified cytoplasmic thymidine kinase activity, different concentrations of nucleotides were added in the presence of 1 mM ATP to the enzyme reaction mixture. The results are shown in Chart 10. Product inhibition can be seen with dTDP and dTTP completely inhibiting the reaction and dTTP inhibiting 50% at equimolar concentrations of ATP. A small amount of inhibition is also seen with dCTP and UMP.

**Effect of Various Pyrimidine Nucleoside Analogs.** Various pyrimidine analogs were tested as inhibitors of purified cytoplasmic thymidine kinase with the results shown in Table 3.
regulate estrogen effects in vivo. Such knowledge might be especially useful in instituting hormonal therapy for mammary cancers. In an effort to elucidate the mechanism of the action of estrogen, we examined its effect on thymidine kinase in MCF-7, a human breast cancer cell line in tissue culture. This is an enzyme in the salvage pathway of nucleotide biosynthesis. A close correlation between thymidine kinase activity and DNA synthesis has been noted in rat pituitary (23), developing rat cerebellum (24, 25), and erythropoietic mouse spleen cells (19). In the case of rat pituitary, estradiol was shown to induce both thymidine kinase and DNA synthesis, without affecting thymidylate synthetase activity (23).

The dose-response and time course of thymidine kinase induction by estradiol in human breast cancer cells indicate that thymidine kinase may be involved in estrogen induction of cell growth. Stimulation of thymidine kinase activity paralleled increases in thymidine incorporation. Tamoxifen, an antiestrogen which inhibited cell growth below control levels, also reduced thymidine kinase activity. The effects on thymidine incorporation by these hormones may not directly measure DNA synthesis. A change in the specific activity of the precursor pool would affect the enzyme levels.

That estrogen is inducing thymidine kinase activity via a mechanism involving binding to specific receptor proteins is suggested by the fact that breast cell lines which lack estrogen receptor do not show stimulation of thymidine kinase activity or thymidine incorporation by estradiol.3 We have shown previously (11) that there is good agreement between concentrations of antiestrogen which compete with estradiol for receptor and inhibitory effects. Furthermore, there is reversal of antiestrogen effect by concentrations of estradiol which are consistent with their relative affinities for estradiol receptor. However, there appears to be about a 10-fold discrepancy between binding and stimulation experiments; i.e., only a fraction of total receptor sites need to be occupied for optimal induction.

The increase seen in the Vm for thymidine while the Km remained constant in the presence of estrogen suggests that more active enzyme is present. We hope to eventually show whether the increase in specific activity of thymidine kinase is due to an increase in the intracellular concentration or to a change in the activity of the enzyme. We are currently attempting to develop breast cancer cells that are deficient in cytoplasmic thymidine kinase by growing them in the presence of bromodeoxyuridine. If cells lacking thymidine kinase activity exhibit altered responsiveness to estrogen, it would support the idea that thymidine kinase activity is involved in estrogen induction of increased DNA synthesis.

In order to further study the hormonal regulation of thymidine kinase in more detail, we decided to purify and characterize it. The purification was done on breast cancer cells grown in the presence of fetal calf serum; hence, they were maximally stimulated by estrogen. This was necessary because of the low level of enzyme present in unstimulated cells under serum-free conditions. With the affinity column technique, we obtained a 2000-fold purification which migrated as one band on a dissociating polyacrylamide gel system and on isoelectric focusing gels. The estimated molecular weight of 177,000 differed from that obtained for other human cell lines by other techniques. Lee and Cheng (8) obtained a molecular weight esti-
mated 90,000 for blast cells of acute myelocytic leukemia using a molecular sieve, while Kit et al. (5) estimated the molecular weight of HeLa S3 (human cervical carcinoma) cells to be 85,000 by the sedimentation coefficient. We have found no evidence that our molecular weight is due to an aggregate, and increasing the concentration of detergents and thiol compounds does not affect our results.

The properties of thymidine kinase isolated from various tissues differ greatly, and other investigators have described several forms of enzyme (5, 8, 15, 17, 21). Taylor et al. (21) compared thymidine kinases isolated from human fetal and adult liver and found that the fetal form resembled the enzyme present in 2 human tumor cell lines in some of its characteristics. When Taylor et al. fractionated and electrophoresed the liver cells, the fetal form was located in the cytoplasm, while the mitochondria contained the faster-migrating adult form of thymidine kinase. The cytoplasmic thymidine kinase in MCF-7 breast cancer cells had characteristics similar to those of human fetal liver enzyme and cytoplasmic enzyme isolated from blast cells of acute myelocytic leukemia. The pH optimum, to be 8.5, 000 by the sedimentation coefficient. We have found using a molecular sieve, while Kit et al. (5) estimated the fetal and cytoplasmic liver enzyme. It also had no effect on the GTP. dCTP has been found to inhibit mitochondria and adult liver and mitochondria were able to utilize CTP, DTP, and (15) reported that, in adult rat adrenal tissue, an induced form would not significantly affect our results. Masui and Garren (1973) reported that, in adult rat adrenal tissue, an induced form of thymidine kinase, the mitochondria of MCF-7 cells were able to utilize only ATP and dATP as substrates. The nucleoside specificity of thymidine kinase may also be useful in studying the mechanism by which estrogen regulates its activity.

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REFERENCES

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