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Lactate Dehydrogenase in Estrogen-responsive Human Breast Cancer Cells[1]

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ABSTRACT

Lactate dehydrogenase activity (LDH) was measured in the MCF-7 human breast cancer cell line derived at the Michigan Cancer Foundation from a patient with metastatic breast adenocarcinoma. LDH was found in the 46,000 × *g* supernatant of cell lysates, but not in the culture medium. Only the fifth isozyme (LDH-5) could be demonstrated by cellulose acetate electrophoresis and relative heat inactivation studies.

When endogenous steroids were removed from the medium, addition of estrogen to the growth medium for several days elevated LDH 2-fold above controls; LDH was not altered when MCF-7 cells were treated with progesterone, hydrocortisone, prolactin, insulin, or triiodothyronine. A physiological concentration (0.1 nM) of 17β-estradiol was sufficient to produce a maximal LDH increase. There were no qualitative isozyme changes in response to estrogen.

LDH activity may therefore be a useful marker protein for studying hormone action in the MCF-7 human breast cancer cell line.

INTRODUCTION

The MCF-7 cell line (a human breast cancer cell line derived at the Michigan Cancer Foundation from a patient with metastatic breast adenocarcinoma) (18) has been used extensively to study the molecular interactions of estrogens and antiestrogens with the estrogen receptor (1, 8–10); presumably, the action of receptor binding to ligand is followed by gene activation and subsequent estrogen-dependent events. Estrogen has been shown to alter growth (11) and progesterone receptor levels (8) in MCF-7 cells. Thus, the MCF-7 cell line appears to offer an opportunity to study, under very defined conditions, the interrelationships of gene regulation and growth control of human breast cancer cells by estrogen.

LDH[3] is well known to be elevated in human neoplastic disease. A recent demonstration that LDH is useful for monitoring human tumor growth in athymic mice suggests a possible role for LDH in analyses of antitumor therapy (15). The potential use of patient tumor patterns of LDH (and other glycolytic enzyme activities) in conjunction with estrogen receptor analysis (13) for selecting breast cancer patients for hormone and/or chemotherapy has also been postulated (17). Goldman *et al.* (4) first demonstrated that total LDH activity was directly related to the degree of cancer of human breast tumors. Hilf et al. (6) more recently showed a 2-fold greater LDH activity in infiltrating ductal carcinoma than in benign proliferative disease and normal breast tissue. They also showed a significant increase in the proportion of the LDH-5 isozyme in neoplasia. Patient sera patterns of LDH have also been reported to have potential prognostic significance in human breast cancer (16). Many human breast tumors have been found to be estrogen dependent; furthermore, estrogen is capable of elevating LDH-5 in mammalian uteri (5). For these reasons it seemed worthwhile to investigate LDH activity as a potential marker of estrogen action in the hormone-responsive MCF-7 human breast cancer cells.

MATERIALS AND METHODS

Cell Cultures. MCF-7 human breast cancer cell line at passage 102 was originally obtained from Dr. H. D. Soule at the Michigan Cancer Foundation. Except for initial passages with 0.125% trypsin:1 mM EDTA, these cells have been subcultured weekly with the use of 1 mM EDTA:HBSS to remove cells from culture vessels. Cells were grown at 37° under a 5% CO_2:95% air, high-humidity atmosphere in Eagle's autoclavable MEM, supplemented with 1% nonessential amino acids, 1% glutamine, 5% calf serum, bovine insulin (6 ng/ml), 0.5% saturated sodium bicarbonate, and gentamicin (25 μg/ml). For experiments, 1,000,000 cells were plated into Corning plastic 75 sq cm T-flasks, with control medium consisting of 20 ml MEM supplemented as above but without insulin and with 5% calf serum stripped (7) of endogenous steroids. Cultures were fluid changed to experimental treatments 24 hr later. Medium changes were made every 2 days until confluence, and then were done daily.

Cell Harvests. At the indicated times, growth medium was aspirated, and the flasks were washed 3 times with an equal volume of warm HBSS and then were incubated with 1 mM EDTA:HBSS for 7 min. Cells were gently shaken off the flasks, suspended, and then one-tenth of the total cell suspension was pelleted and frozen at −20° to await DNA determinations. The remaining cells were sedimented at 500 × *g* for 7 min. These pellets were resuspended in 2.0 ml of 0.9% NaCl, solution and then were rapidly frozen at −70° until use. Cell suspensions were slowly thawed, vortexed, and then centrifuged at 46,000 × *g* for 1 hr. The supernatants (cell lysates) were then assayed for total LDH activity and/or used for cellulose acetate electrophoretic separation of LDH isozymes. Microscopic observation showed

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[3] The abbreviations used are: LDH, lactate dehydrogenase (L Lactate:NAD^+ oxidoreductase, EC 1.1.1.27); HBSS, Ca^{2+}-Mg^{2+}-free Hanks' balanced salt solution; MEM, minimal essential medium.

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that 100% of the cells were ruptured, as indicated by trypan blue dye uptake.

DNA and Protein Quantitation. Frozen cell aliquots were thawed, hydrolyzed in 0.56 N perchloric acid, and then assayed for DNA content according to the diphenylamine method of Burton (2). Protein content of the cell lysates was measured according to the method of Lowry *et al.* (12), using bovine serum albumin as the standard.

LDH Assay. The LDH assay was performed according to the method of Wacker (19), as modified by Calbiochem (Los Angeles, Calif.). The conversion of L-lactate to pyruvate was measured at 28° with the use of Calbiochem reagents and control human sera standards. Initial readings were made 30 sec after addition of the sample to the reaction mixture, and a final reading was taken 2 min later. Experimental samples were diluted with 0.9% NaCl solution to give activities yielding an absorbance change at 340 nm less than 0.200.

Electrophoresis. LDH electrophoresis was performed on cellulose acetate with the use of the Dade Iso-Form System (Dade Division, American Hospital Supply Corporation, Miami, Fla.), which is a modification of the staining procedure originally described by Opher (14). Also used were barbital buffer (pH 8.6; ionic strength, 0.075), Beckman Microzone electrophoresis system, and a scanning densitometer (Helena Laboratories Corp., Beaumont, Texas).

Chemicals. Calf serum, nonessential amino acids, glutamine, and MEM were purchased from Grand Island Biological Co., Grand Island, N. Y. Bovine insulin, 3,3', 5-triiodo-L-thyronine, and progesterone were purchased from Sigma Chemical Co., St. Louis, Mo. and 17β-estradiol, hydrocortisone, and LDH assay reagents were purchased from Calbiochem. Prolactin (NIH-P-S-12 ovine) was donated by National Institute of Arthritis, Metabolic, and Digestive Diseases (Bethesda, Md.).

RESULTS

We first determined optimal assay conditions and the characteristics of the LDH reaction with the use of either MCF-7 cell lysates or standard human serum (Caltrol II) as depicted in Chart 1. Regardless of the rate, the reaction is linear up to an absorbance change of 0.250. Consequently, all experimental samples were diluted to maintain linear kinetics.

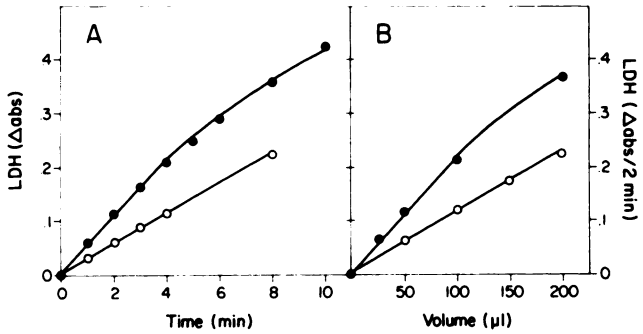

Chart 1. Characteristics of the LDH assay. The change in absorbance (Δ*abs*) due to the reduction of NAD^+ was monitored at 340 nm. The reaction mixture contained 0.07 M L-lactate, 3.5 mM NAD^+, and 0.05 M glycine buffer, pH 8.8. The time courses (*A*) began 30 sec after addition of 50 μl of sample to 3 ml of reaction mixture. Identical conditions were used to assay the effect of sample volume (*B*) on the LDH assay. ●, values obtained with lysates of MCF-7 cells grown on experimental medium as described in the text; ○, data from a human serum standard (Caltrol II).

Human serum and MCF-7 cell lysates were heated at 65° for 35 min, a treatment to which LDH-1 is known to be resistant (20). This treatment totally eliminated LDH activity in cell lysates, while some activity was retained in serum that is a mixture of isoenzymes (Table 1). Mixtures of lysate and serum gave the results expected from simple dilution. We find that levels of LDH activity in untreated MCF-7 cell lysates were routinely 100- to 1000-fold greater than in the standard human sera when they are expressed per mg protein. LDH activity was not detectable in the cell culture medium.

Since the heat inactivation suggested that LDH-1 was not present in cell lysates, it became necessary qualitatively to characterize the enzyme activity in the cell lysates. The cellulose acetate electrophoretic pattern shown in Chart 2 illustrates the form of LDH present in MCF-7 cells. Only the LDH-5 isoenzyme appears to be present.

Although it had no effect on the qualitative electrophoretic pattern, estradiol more than doubled LDH activity above untreated cells (Table 1; Chart 3). Estrogen-stimulated LDH activity was apparent only after 3 or 4 days of treatment, regardless of whether cells were in logarithmic or confluent phases of growth. Chart 4 shows that at 5 days of treatment maximal responses were obtained with concentrations of 0.1 nM or greater. Other natural estrogens, estrone and estriol, similarly stimulated LDH activity (data not shown). However, LDH was not altered in cells treated for 10 days with progesterone (0.1 nM to 1.0 μM), hydrocortisone (10 nM), triiodothyronine (0.1 nM or 1.0 μM), prolactin (0.4 nM to 0.04 μM), or insulin (0.01 nM to 0.1 μM).

DISCUSSION

LDH is an ubiquitous enzyme that functions in a regulatory manner at a key point in normal intermediary metabo-

Table 1

Relative heat inactivation study of LDH activity in MCF-7 cell lysates, a human serum standard, and combinations

Total LDH (lactate-to-pyruvate conversion) was measured by the method of Wacker (19), as modified by Calbiochem. Assays were run at 28°. Each sample (200 μl) was heated at 65° for 35 min to inactivate all but the LDH-1 isozyme (20). Control samples were not heated, but they were identical otherwise. Combinations were assayed from a mixture of 100 μl of each component. MCF-7 cells grown for 10 days on medium containing calf serum stripped of endogenous steroids (SCS) with or without added 17β-estradiol (10 nM) were lysed by a freeze-thaw step and then were centrifuged at 46,000 × *g* for 1 hr. The supernatants were then assayed for LDH as indicated.

	LDH (ImU/ml)	
Sample	Control	Heated
MCF-7 cell lysates		
Untreated control	602	0
Estradiol treated	2030	0
Human serum standard[a]	362	65
Untreated lysate:serum (1:1)	567	38
Estradiol-treated lysate:serum (1:1)	1395	34

[a] Caltrol II, shown in our laboratory by electrophoresis to have isozymes 1, 2, 3, 4, and 5 in the percentages of 17, 22, 19, 10, and 32, respectively.

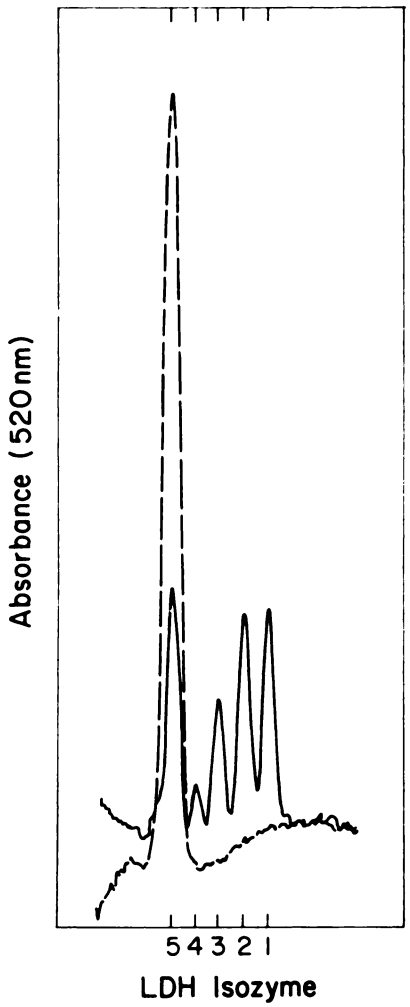

Chart 2. Cellulose acetate electrophoretic patterns of LDH isoenzymes. ——, relative distribution of isozymes from the serum of a patient with apparent myocardial infarction; - - - -, isozyme pattern of MCF-7 cell lysates. These patterns are not quantitatively comparable, but they do show the form of isozyme present in each sample. Cell culture methods are explained in the text.

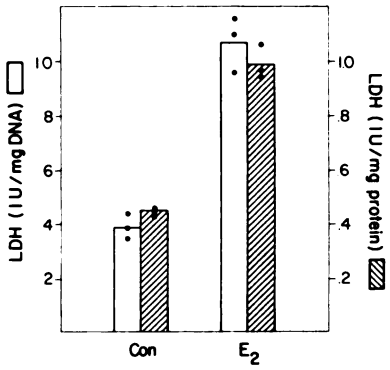

Chart 3. Effect of estrogen on LDH activity in MCF-7 cells. Cells were grown for 10 days with (E_2) or without (*Con*) 10 nM 17β-estradiol prior to harvest at confluence. The control growth conditions, harvest methods, and LDH assay are described in "Materials and Methods." The *bar* height represents the mean of LDH values (●) for triplicate flasks, expressed per mg protein and per mg DNA.

lism. LDH is a tetrameric molecule usually represented by 1 of 5 possible isoenzymes (3). The activity and form of LDH in MCF-7 cells has not been reported elsewhere and was

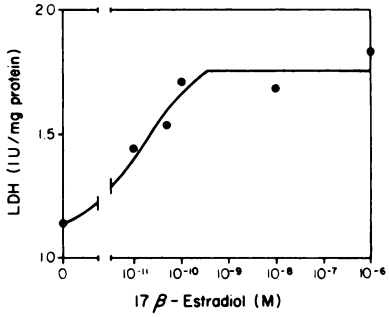

Chart 4. Dose response of the LDH activity in MCF-7 cells to estrogen. Cells were grown to confluence and then were exposed to 17β-estradiol at the indicated concentrations for an additional 5 days. ●, mean of measured values for duplicate flasks. Growth conditions and the enzyme assay are described in "Materials and Methods."

thus characterized as a prerequisite to determining its potential usefulness as a marker protein of estrogen action.

We find (*a*) that MCF-7 cells have very high levels of LDH activity, (*b*) that only the fifth isoenzyme is present, and (*c*) that estradiol stimulates LDH levels. The significance of the high levels and the form of LDH in MCF-7 cells is uncertain. These levels are higher than those found in serum, even in disease states, but are in the range reported for normal or malignant breast tissues (6). Total LDH in infiltrating ductal carcinoma was reported to be 2-fold that of normal breast, fibrocystic disease, or fibroadenoma (6). Whether elevated levels are principally due to increased synthetic rates or to decreased intracellular metabolism or slowed excretion rates is unknown.

Also potentially of interest in human breast cancer is the demonstration that only the fifth isoenzyme of LDH is present in MCF-7 cells. Several investigators have reported an elevation in the proportion of LDH-5 in human breast neoplasia compared to that in normal breast tissue (4, 6, 16), and estradiol increases the rate of LDH-5 synthesis in uteri of immature rabbits and rats (5).

The search in MCF-7 cells for specific and useful markers of estrogen action that lie distal to the binding of estrogen to its receptor molecules and subsequent gene activation has met with some success. Progesterone receptor has been shown to be such a product (7, 8). LDH itself has the potential to control metabolic functions crucial to growth and may thus prove to be quite useful.

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