Modulation of Alkaline Phosphatase Activity in Cell Cultures Derived from Human Urinary Bladder Carcinoma

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SUMMARY

T24 cells derived from a human urinary bladder carcinoma have very low alkaline phosphatase activity. The specific activity is 500 to 1000 times lower than that of HeLa S3 cells. Enzyme activity increases when T24 cells are grown in the presence of prednisolone (Δ1-hydrocortisone). Increase in the osmolality of the culture medium with NaCl or sucrose has a similar effect. When cells are grown in hyperosmolar medium with prednisolone added, a synergistic effect is noted. Neither stimulus significantly affects acid phosphatase activity.

INTRODUCTION

Diverse metabolic and enzymatic properties of benign and malignant mammalian cell lines have been elucidated by the use of tissue culture systems. Previous studies have shown that the alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) activity in some heteroploid cells of human origin is modulated by corticosteroids (3), osmolality (11), and the concentration of human serum (7) in the culture medium. Reports on long-term cultures derived from human urinary bladder cancers are scant, since only recently have several such cell lines been established (1, 12). This report concerns the alkaline phosphatase activity in T24, a heteroploid cell line initiated by Bubenik et al. (1) from a histologically verified urinary bladder carcinoma Grade III. This line is characterized by a tumor-specific antigen (1).

MATERIALS AND METHODS

Cell line T24 was obtained through Dr. Carol O'Toole from the Department of Immunology, Wenner-Gren Institute, Stockholm, Sweden, and from Dr. Jørgen Fogh, Sloan-Kettering Institute, N. Y. The cells were grown as monolayers in milk-dilution bottles. BME supplemented with 15% fetal calf serum, penicillin (100 units/ml), streptomycin (100 µg/ml), and amphotericin B (0.25 µg/ml) was routinely used. In some experiments, the cells were cultured in MEM + 10% calf serum, Medium 199 + 15% fetal calf serum, and McCoy's medium + 15% fetal calf serum. The osmolality of the medium was increased by the addition of NaCl or sucrose 24 hr following cell transfer. Prednisolone (Δ1-hydrocortisone) was dissolved in ethanol (100 µg/ml), and appropriate amounts of this solution were added to the cultures 24 hr after cell transfer. Ethanol was added to the controls. In some experiments, NaCl or sucrose and prednisolone were added simultaneously.

After growth in an atmosphere of 5% CO2 in air at 37° for 7 days, the cells were dispersed with 0.25% trypsin in balanced salt solution. The cells from several bottles were combined, washed twice with and suspended in cold 0.15 M NaCl, and then stored at -20°. Homogenized preparations of disrupted cells were obtained by ultrasonic treatment with an ice-water-cooled Raytheon 10-kc oscillator (6). Alkaline phosphatase activity was determined by the hydrolysis of p-nitrophenyl phosphate, with the use of 1 M 2-amino-2-methyl-1-propanol-HCl buffer, pH 10.6, at 38° (6). Acid phosphatase activity was assayed with the same substrate at pH 4.8, with 0.1 M acetate buffer. Duplicate assays agreed with less than 10% variation. Enzyme activity was related to the protein content determined according to the method of Lowry et al. (9). Specific activity of alkaline phosphatase was expressed as nmole of p-nitrophenol liberated per 30 min per mg of protein at 38°, and that of acid phosphatase was expressed as µmole of p-nitrophenol liberated per 30 min per mg of protein at 38°.

RESULTS

The base-level alkaline phosphatase activity of the human bladder cancer cell line T24 was extremely low, even when compared to the specific activity of lines falling within the category of cells with low activity (11), such as HeLa S3 or Zimmer liver 4A. Thus, specific activity of T24 at the end of a 7-day growth cycle in BME + 15% fetal calf serum ranged from 0.7 to 1.7. These values are 500 to 1000 times lower than the specific activity of HeLa S3. Similar low basal levels were also found when T24 was grown in 3 other media (see below). The low alkaline phosphatase activity of T24 cannot be ascribed to the effect of trypsin during the harvesting procedure, since equivalent results were obtained when the cell monolayers were dispersed with a rubber policeman. Furthermore, the low levels of activity were qualitatively confirmed by an agar-p-nitrophenyl phosphate overlay technique (10), with cells attached to the...
growth surface. In contrast, the levels of acid phosphatase activity in T24 cells were within the range observed with other heteroploid lines of human origin (5, 7).

Specific activity of alkaline phosphatase of T24 cells was significantly increased when prednisolone (Δ4-hydrocortisone) was added to the culture medium (Table 1). Since the effect of the steroid hormone was maximal at 0.5 μg/ml (1 μM), this concentration was used in all subsequent experiments. Prednisolone did not affect acid phosphatase activity. The observed drop in total cell protein content can be ascribed to the inhibition of cell proliferation by ethanol in which prednisolone was dissolved.

The specific activity of alkaline phosphatase in T24 cells was also increased by the addition of NaCl to the culture medium. Whereas no effect was noted by adding 5 or 10 mM NaCl, the specific activity was twice that of control cultures with 20 mM and, at higher concentrations of NaCl, the activity was increased further (Table 2). No significant diminution of the total cell protein content was noted when the culture medium was supplemented with small amounts of NaCl, but concentrations higher than 60 mM markedly inhibited cell proliferation. The largest increases in specific activity were noted with the highest concentrations of NaCl, but the significance of these findings is not clear. The possibility exists that the increase in alkaline phosphatase activity under these conditions represents an effect secondary to inhibition of cell proliferation. However, when cells were grown in the presence of puromycin (11) or when relatively large amounts of 2% ethanol were added to the medium, as in the control cultures of T24 cells growing with prednisolone, no increase in alkaline phosphatase activity was observed. NaCl had little effect on acid phosphatase, but at high concentrations the specific activity tended to be slightly lower than in controls. The increase in alkaline phosphatase activity appeared to be due to an osmotic rather than an ionic effect (11), because supplementation of the medium with sucrose (which is not metabolized by mammalian cells in vitro) also caused elevation of enzyme activity. Thus, when T24 cells were grown in the presence of 80 mM sucrose, alkaline phosphatase activity was 14.7, compared with 1.4 in controls.

<table>
<thead>
<tr>
<th>Prednisolone added to culture medium (μg/ml)</th>
<th>Alkaline phosphatase*</th>
<th>Acid phosphatase*</th>
<th>Cell protein (mg/flask)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.7</td>
<td>1.45</td>
<td>1.16</td>
</tr>
<tr>
<td>0.5</td>
<td>14.7</td>
<td>1.75</td>
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<td>0.75</td>
<td>8.7</td>
<td>1.81</td>
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<tr>
<td>1.0</td>
<td>4.2</td>
<td>1.60</td>
<td>0.90</td>
</tr>
<tr>
<td>2.0</td>
<td>3.7</td>
<td>1.58</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* Specific activity expressed as nmoles of p-nitrophenol liberated per 30 min per mg protein at 38°C.

In contrast to the additive increase in alkaline phosphatase activity noted when HeLa S3 cells were grown in hyperosmolar medium containing prednisolone (11), the response by T24 to the simultaneous addition of steroid hormone and NaCl was of a synergistic nature. The specific activity of T24 cells grown in hyperosmolar medium (403 mOsmoles/kg) with prednisolone added was up to 200 times higher than in control cultures (Table 3). Similar results were obtained when sucrose and steroid were added simultaneously. The increase in alkaline phosphatase activity caused by hyperosmolality and/or prednisolone was usually discernible within 24 hr after the addition of the stimuli, and the effect was independent of the culture medium used. As shown in Table 3, a qualitatively similar response was seen when T24 cells were grown in stimuli-containing MEM, Medium 199, or McCoy’s medium. No significant changes in the activity of acid phosphatase were noted in these experiments.
DISCUSSION

As the foregoing results show, the basal levels of alkaline phosphatase activity in T24, a cell line derived from a human urinary bladder carcinoma, is extremely low, whereas acid phosphatase activity is within the range found in other human cells, irrespective of their alkaline phosphatase levels (5, 7). Like the enzyme of HeLa S3, the alkaline phosphatase of T24 is modulated by corticosteroids and by hyperosmolality. A significant increase in activity is noted following growth in the presence of prednisolone or in media made hyperosmolar with NaCl or sucrose. However, the effect of increasing the osmolality in the presence of steroid in T24 is markedly different from that in HeLa S3 cells. Whereas, in the latter, an additive effect is usually obtained (11), with T24 a synergistic action is always observed and this response is independent of the basal culture medium used.

The mechanisms responsible for the control of alkaline phosphatase activity in cultured cells by hyperosmolality or by hormones have not been elucidated. Studies with HeLa cells have suggested that the hormonally induced increase in activity is not due to an increase in the rate of enzyme synthesis or a decrease in the rate of enzyme degradation (2). It appears that the hormone acts by initiates the synthesis of a modifier molecule that interacts with alkaline phosphatase to produce an enzyme with enhanced catalytic efficiency, but that is otherwise indistinguishable from the base-level enzyme. This type of regulation differs from the model of induction of tyrosine aminotransferase in rat hepatoma cell cultures in which the inducing steroid is thought to antagonize the action of a posttranscriptional repressor (20).

Although the importance of osmolality has been emphasized since the advent of cell culture techniques (21), few studies have dealt with osmolality changes in relation to particular metabolic and enzymatic properties of cells in culture (4, 13, 15, 19). A representative example of such investigations is the adaptation of Ehrlich ascites tumor cells to grow in hyperosmolar medium, conditions under which the cells display increased ATPase activity (15). Since it might be relevant to an understanding of the mode of action of hyperosmolality and prednisolone, the recent work by Rübner and Hövel (14) is mentioned. These investigators, by using carrier-free electrophoresis, found that heteroploid cells (S111b) grown in hyperosmolar medium (430 or 540 mOsmoles) have higher migration rates toward the anode than controls, thus indicating that the membranes of experimental cells possess an increased negative charge (14). Because it is well known that cell-surface sialic acid is responsible for the electrokinetic behavior of cells in an electrolyte medium, it could be postulated that S111b cells grown under hyperosmolar conditions have elevated levels of sialic acid. If this interpretation is correct, it would represent the first common action (other than alkaline phosphatase) shared by hyperosmolality and prednisolone, since it has been shown that in HeLa cells the steroid promotes the accumulation of increased levels of sialic acid by repressing the formation of a specific sialopeptidase (17).


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