CYCLIC ALTERATION OF ELECTROPHORETIC PROPERTY ASSOCIATED WITH GROWTH CIRCLE IN HeLa S3 CELLS

BY

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ABSTRACT

Changes of electrical cell-surface charge associated with cell cycle were examined electrophoretically in the synchronous culture of HeLa S3 cells. The electrophoretic mobility of the cells increased abruptly in the mitotic phase as high as 45% compared with that of cells in the S phase. This difference of mobility was obtained by comparing pure population of mitotic cells and S-phase cells. Mitotic cells were also found to be more susceptible to neuraminidase than S-phase cells in term of electrophoretic mobility, a greater amount of sialic acid being correspondingly released from the former. The mechanism of the electrophoretic change associated with the process of cell cycle is discussed, with special reference to the distribution of electric charge in the cell surface membrane.

INTRODUCTION

It has been reported that the malignant transformation of cells is associated with the cell surface changes, one of which is an increase in charge density. Purdom et al. demonstrated a correlation between the stepwise progression of a mouse sarcoma and the corresponding increase in the surface charge density of its individual cells. They further stated that electrophoretic mobility of the cells is associated with the malignant transformation, but also with proliferation of the cells.

On the other hand, cells of certain types derived from the embryonic or regenerating normal tissue have a higher net negative electrophoretic charge than the cells from the corresponding adult homologous tissue. The higher electrophoretic mobility appeared to be associated with normal cell growth process. Furthermore, several authors have reported that there is no significant difference in electrophoretic mobility between some malignant strains of fibroblastic cell origin and their normal antecedent cell cultured in vitro.

Thus, for elucidating the increase of electrophoretic mobility observed in the malignant cells, an analytical study on the changes of the electric cell-surface properties associated with the cell growth process should be required. In the present work, an attempt was made to ascertain whether the electrophoretic mobility of HeLa S3 cells may be altered to a greater or lesser extent during the cell cycle, employing the synchronous culture system.

MATERIALS AND METHODS

Cells—HeLa S3 cells were used throughout the present experiments. The culture
medium used was Eagle's minimum essential medium containing 100 units/ml of penicillin and 100 μg/ml of streptomycin, and supplemented with 10% calf serum.

**Synchronous culture**—To the cells which had been growing exponentially for 3 days, excess thymidine was added to give a final concentration of 2.5 mM. After cultivating for 24 hr, the cells were washed with Dulbecco's phosphate-buffered saline and then cultured with a fresh culture medium. Thus, almost all the cells were simultaneously initiated to grow soon after the release from excess thymidine, and were assumed to enter the S phase. In this synchronized culture, the electrophoretic mobility of the individual cells, mitotic index, and the percentage of S-phase cells were examined every 2 hr.

**Selective collection of both mitotic cells and S-phase cells**—To collect the cells in the mitotic phase, 2×10⁶ cells were inoculated in Roux bottles with 60 ml of the culture medium. After 2 days of incubation, 2.5 mM thymidine was added, this concentration being maintained for 24 hr to synchronize the cell cycle. Thereafter, the medium was renewed and the culture was further incubated for 12 hr. Colcemid (Ciba, Switzerland) was then added to the culture in a final concentration of 0.015 μg/ml. After incubation with colcemid for 2 hr, the culture bottles were shaken gently to dislodge the mitotic cells selectively, according to the method reported by Tarasima and Tolmach. In some experiments, the mitotic cells were also collected by gently shaking the bottles without pre-addition of excess thymidine and colcemid.

The cells in the S phase, 2×10⁶ cells suspended in 60 ml of the medium were seeded in Roux bottles and, after incubation for 4 days, thymidine was added to a final concentration of 2.5 mM. After 24 hr the medium containing thymidine was discarded and a fresh culture medium was added. The cells incubated for 3 hr were used as the S-phase cells. In a part of the experiments, S-phase cells were also prepared by 16-hr incubation with 10⁻⁶ M amethopterin in place of 24-hr incubation with 2.5 mM thymidine.

**Estimation of the percentage of S-phase cells and mitotic cells**—The percentages of cells synthesizing DNA were determined every 2 hr by pulse-labeling for 1 hr with H³-thymidine in cultures. The cultured cells were fixed with acetic acid-ethanol. The emulsion film (Kodak, U.S.A.) was mounted on labeled cells and exposed in a sealed box for a period ranging from 1 to 2 weeks. After developing the autoradiographic film and staining the cells with Giemsa's solution, the percentage of cells containing silver grains was determined, counting about 2,000 cells. The background grain was usually negligible. The cells with 10 or more grains were estimated as the S-phase cells. The percentage of mitotic cells was also determined on the morphological basis, counting about 2,000 cells.

**Electrophoretic measurement**—The cells were washed with physiological saline and suspended in standard medium of Michaelis' Veronal buffer, pH 7.0, with the concentration of cells 2×10⁶ cells/ml. The cell suspension was injected through a slender polyethylene tube into the visual field of a conventional chamber, which had been filled previously with the buffer solution. Migration of each cell in both directions was timed by reversing the direction of the electric current alternately, and every mean value obtained by measurement in both directions was determined for each cell. In the measurement of the cells at each growth phase, the mobility was
checked on 20 or more cells at 25°±0.2°C and their mean value with measurement errors are given.

*Calcium absorption test*—In addition to the usual electrophoretic measurement, mobility of the cells suspended in Michaelis’ Veronal buffer containing CaCl₂ at a concentration of 10 mM was measured, based on the preliminary experiments of Yamada and Amano[12]. Difference in the mobility of the cells suspended in the medium with and without CaCl₂ was calculated. Hence, it became possible to determine the decrease in the charge attributable to absorption of calcium ions on the cell surface.

*Neuraminidase treatment and sialic acid assay*—Neuraminidase (receptor-digesting enzyme) purified from a culture filtrate of *Vibrio cholerae* was purchased from Burroughs Wellcome & Co., London. The cells were suspended in buffered saline (80% of 0.154 M NaCl, 20% of 0.1 M acetate buffer, pH 5.6) containing 0.1 mM of CaCl₂ and enzyme solution (30 units of neuraminidase/0.1 ml of wet cell volume) and then incubated for 30 min. After the treatment, the cells were washed by centrifugation with 0.154 M NaCl solution. The neuraminidase treatment under the present conditions results in 10±0.5% reduction in electrophoretic mobility of rat erythrocytes (standard cells). After the neuraminidase treatment, the amount of sialic acid (N-acetylmuramic acid) released into the supernatant of the cell suspension was also measured by Warren’s colorimetric method[13], assuming that all of the neuraminic acid released is N-acetylmuramic acid.

**RESULTS**

*Follow-up examination of the electrophoretic mobility of HeLa S3 cells during synchronous culture*—As shown in Fig. 1, the electrophoretic mobility varies with time after the removal from excess thymidine and reaches a maximum when the percentage of mitotic cells is highest and reaches a minimum when the percentage of the S-phase cells is highest. The mean mobility begins to increase 4 to 8 hr after the release from excess thymidine, rises sharply to a peak 10 to 12 hr after the release, and falls 14 to 18 hr after the release. The mean mobility of the cells during the mitotic peak is about 25% greater than that of cells at 2 hr after the release from excess thymidine, while the mitotic indices in the former and latter are 16 and 3%, respectively. An inverse relationship is observed between the mobility and the percentage of ³H-thymidine labeled cells.

*Difference in the electrophoretic mobility between pure mitotic cells and S-phase cells*—Three samples of mitotic cell population were separately collected, the mitotic indices of them being 41, 40 and 37%. Each 0.2 to 0.5 ml of these samples was inserted into the electrophoretic chamber and only mitotic cells definitely identified in the visual field of the microscope for electrophoresis were chosen for measuring the electrophoretic mobility (as shown in Fig 2). Since all the cells measured were mitotic cells, their electrophoretic mobilities are those of the pure mitotic population. On the other hand, cell samples of the S-phase cell population obtained by the present method were estimated to contain more than 80% S-phase cells (see Fig. 1). By using these cell samples, electrophoretic mobilities of mitotic cells and S-phase cells were estimated (Fig. 3). The mean mobility of mitotic cells was 1.278±0.014 μm/sec/V/cm, while that of S-phase cells was 0.882±0.013 μm/sec/V/cm. This result indicated the significant difference of as much as 45%
Fig. 1. Changes in electrophoretic mobility (○—○—), percentage of mitotic cells (△—△—), and percentage of S-phase cells (□—□—), of HeLa S3 cells, with time after release from excess thymidine. Mitotic cells were determined on the morphological basis and S-phase cells were determined by pulse-labeling for 1 hr with H³-thymidine. Insert: Distribution of mobility of HeLa-S3 cells released from excess thymidine at the indicated time.

Miotic cells

1.36 1.30 1.30 1.28 1.24 1.23 1.21 1.20 1.18 1.18 1.12 1.10

S-phase cells

1.04 1.03 0.92 0.92 0.89 0.85 0.85 0.85 0.85 0.84 0.81 0.80

Fig. 2. Electrophoretic mobilities of individual cells in mitotic and S phases, examined by the autophoto-recording cell electrophoretic apparatus²⁰. The number under each cell shows the electrophoretic mobility (µm/sec/V/cm).
in the mean mobility between mitotic cells and S-phase cells. The highest mobility of the cell among the total mitotic cells estimated was as high as 1.52 μm/sec/V/cm. Different responses to neuraminidase treatment were also observed between the cell samples in the mitotic and S phase. Reduction in electrophoretic mobility induced by neuraminidase treatment were 24.5% and 13.6%, respectively, in mitotic and S-phase cells. While no marked difference was observed in the distribution patterns of electrophoretic mobility of mitotic cells before and after neuraminidase treatment, distribution pattern of S-phase cells became narrow by neuraminidase treatment.

Sialic acid released from the cell surface of both the mitotic and S-phase cells was measured by the colorimetric method under equivalent conditions in which the cells were treated with neuraminidase for electrophoretic examination (Table 1). Though different amounts of cells were employed each time for each assay, the total sialic acid released was in proportion to the total cell number in samples in the same phase. The amount of sialic acid per cell released from the mitotic cells was about twice that from S-phase cells. The difference in release of sialic acid corresponds to the different rates of reduction in electrophoretic mobility of the cells in both phases after neuraminidase treatment. No difference was observed in the ability of calcium absorption between mitotic cells and S-phase cells.

To ascertain whether thymidine, colcemid, or both of them modify the electric charges on the cell surface, electrophoretic mobility of the cells in S-phase cell populations synchronized in two different ways (excess thymidine or amethopterin treatment) were compared with each other. Furthermore, mobilities of the two kinds of mitotic cells collected with or without colcemid were also compared. As shown in Table 2, there was no significant difference (P>0.6) between two kinds of S-phase cells in their electrophoretic mobilities which were measured by the usual method. The mobilities of two kinds of S-phase cells after neuraminidase treatment or after calcium
Table 1. Amount of sialic acid released from HeLa S5 cells in mitotic and S phase

<table>
<thead>
<tr>
<th>Cell</th>
<th>Experimental No.</th>
<th>Cell number $\times 10^8$</th>
<th>Sialic acid $\mu{}$g</th>
<th>Sialic acid/cell $\times 10^{-7}$ $\mu{}$g/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitotic cell</td>
<td>1</td>
<td>74.4</td>
<td>2.0</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>65.3</td>
<td>1.7</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>32.6</td>
<td>1.2</td>
<td>3.7</td>
</tr>
<tr>
<td>S-phase cell</td>
<td>1</td>
<td>184.0</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>92.0</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>161.0</td>
<td>1.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table 2. Electrophoretic mobility of mitotic cells collected with or without colcemid and of S-phase cells synchronized with excess thymidine or amethopterin

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Treatment</th>
<th>Mean electrophoretic mobility ± S.E.$^1$(µm/sec/V/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Not-treated After neuraminidase treatment After calcium absorption</td>
</tr>
<tr>
<td>Mitotic</td>
<td>Colcemid (+)</td>
<td>1.278±0.014 0.962±0.015</td>
</tr>
<tr>
<td></td>
<td>Colcemid (−)</td>
<td>2.396±0.022 0.967±0.012</td>
</tr>
<tr>
<td>S phase</td>
<td>Thymidine</td>
<td>0.882±0.013 0.762±0.009</td>
</tr>
<tr>
<td></td>
<td>Amethopterin</td>
<td>0.937±0.017 0.762±0.016</td>
</tr>
</tbody>
</table>

1) Standard error in measurements.

absorption were not significantly different ($P>0.4$). There was also no significant difference between two kinds of mitotic cells collected with or without colcemid in their electrophoretic mobilities. The difference between two kinds of mitotic cells in the susceptibility to neuraminidase and ability of calcium absorption were scarcely noticeable ($P>0.3$). These results imply that any direct effect or toxicity of thymidine and colcemid on electrophoretic mobility may be negligible.

**DISCUSSION**

In the present paper, it has been clearly shown that the electrophoretic mobility of HeLa S5 cells increases rather abruptly in the mitotic phase as much as 45% compared with that of S phase. The cyclic changes of the electrophoretic mobility during the growth cycle are shown in a scheme of Fig. 4. The magnitude of the changes in mobility of mitotic cells was calculated based on the experiments employing pure mitotic cell populations and S-phase cell populations. Though Mayhew$^{14}$ tried to collect mitotic cell samples by using the synchronous culture for electrophoretic studies, the percentage of mitotic cells in his samples remained at only 16%, and difference in the electrophoretic mobility between cells in the mitotic and S phase was 21%. It is emphasized that only the mitotic cells which can be dis-
Fig. 4. Schematic diagram of electrophoretic changes during the cell cycle of HeLa S3 cells. Abscissa: periods of four cell growth phases (G1, S, G2, and M). Electrophoretic mobility of cells in G1, G2, and M phases were compared with that of cells in S phase.

tinguished from the cells of the other growth phase (see Fig. 2) were measured in the present experiments. This procedure is particularly important for obtaining the present results.

The cyclic pattern of changes in electrophoretic mobility of the cells during the growth cycle is similar to the pattern of changes in membrane potential\(^{15}\), reactivity of cells to osmotic shock\(^{16}\), and conditions of antigenic substances exposed on the cell surface membrane\(^{17,18}\), which also vary with the cell cycle. Moreover, as it is usually seen in cinematograph, active mobility of cells, i.e., bubbling, occurs just before and during the mitotic phase, reaching a maximum when the cells divide into daughter cells. This phenomenon may also suggest abrupt changes in the cell membrane during the mitotic phase.

In the previous paper\(^{19}\), concanavalin A was found to induce biplanar changes in the electrophoretic mobility of rat ascites hepatoma cells. A low concentration of concanavalin A not sufficient to induce agglutination of cells, caused an increase in the electrophoretic mobility, whereas a high concentration of concanavalin A induced a decrease in the mobility. When the cells were treated previously with neuraminidase, the increase in the electrophoretic mobility caused by a low concentration of concanavalin A was considerably accelerated. Furthermore, those cells exhibiting a marked increase in electrophoretic mobility with a low concentration of concanavalin A were also found to be more susceptible to neuraminidase than the untreated control. Therefore, the increased mobility induced by concanavalin A was due to sialic acid molecules on the cell surface membrane, attaching the terminal position of glycoproteins and glycolipids. These glycoproteins and glycolipids, originally situated at deeper sites, may of the cell surface membrane shift reactively into the hydrodynamic shear.

In the present experiments, reduction in the mobility of mitotic cells was induced by neuraminidase treatment in about twice that of the S-phase cells. If the present results and previous evidence mentioned
above are considered together, the electrophoretic changes associated with the cell cycle will presumably occur by a similar process as cited for the effect of concanavalin A on the cells; the transient deviation of the electrophoretic mobility prior to or during cell division may be assumed to be induced by conformational changes of the terminal charged molecules in the surface membrane. In the present experiments, much release of sialic acid was noted in the mitotic cells, corresponding to the rate of reduction in mobility. It is, however, already known that the cellular electrophoretic surface and those surfaces which bear sialic acid molecules sensitive to neuraminidase are not always identical.

References


