CELL CYCLE VARIATIONS IN HELA 65

PLASMA MEMBRANE ALKALINE PHOSPHATASE

Shannon W. Lucid and Martin J. Griffin

Oklahoma Medical Research Foundation
Oklahoma City, Oklahoma 73104

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SUMMARY

HeLa plasma membranes from M, G_1, and S phase cells were isolated from growing synchronous cell cultures. It was found that the specific activity of plasma membrane alkaline phosphatase was over three times higher in the M phase cell than in the G_1 and S phase cell. However, sodium dodecyl sulfate (SDS) polyacrylamide disc gel electrophoresis showed that the S phase plasma membrane contained 5.5 times more alkaline phosphatase protein than did the plasma membrane from mitotic cells, and 11.0 times more than the G_1 phase plasma membrane. This would indicate that the high specific activity in mitosis was due to modification of the alkaline phosphatase protein resulting in increased enzymatic activity.

INTRODUCTION

The membrane of a cell is known to change as a function of the cell cycle. There are changes in antigenicity (1,2), in lectin binding (3), in transport (4), and in the number and shape of microvilli (5). Similar cell surface changes have been observed also in comparing asynchronous populations of normal and transformed cell counterparts (6). The biochemical basis for these changes has not been determined due to the difficulty of obtaining sufficient plasma membranes at various points in the cell cycle for analysis. This biochemical information is necessary to determine variations of plasma membrane components as a function of the cell cycle in order to make valid comparisons between membranes of normal and transformed cells.

MATERIALS AND METHODS

Synchronous Cell Selection: HeLa 65 cells were grown in monolayer culture in Eagle's MEM (7) without calcium supplemented with 10% calf serum and 50 µg/ml gentamicin in plastic T-150 flasks (Corning Co.). To obtain mitotic cells, fifty flasks were each inoculated with 8 million HeLa 65 cells in 50 ml media and grown for sixty hours at 37°. Mitotic cells were selected during no more

TABLE 1

SPECIFIC ACTIVITY OF ALKALINE PHOSPHATASE IN VARIOUS CELLULAR FRACTIONS AS A FUNCTION OF THE CELL CYCLE

Position in Cell Cycles	Specific Activity* Alkaline Phosphatase in Cell Fractions: Plasma Membrane	Cytosol	Nuclear	No. Different Cell Cycle Experiments
M	7.2 ± 1.0	0.90 ± 0.2	1.5 ± .1	7
G_1	2.2 ± 0.3	0.89 ± 0.2	1.2 ± .2	4
S	2.3 ± 0.6	0.75 ± 0.1	1.3 ± .2	6

* Specific Activity is nmole p-nitrophenylphosphate hydrolyzed/min/mg protein

than a 20 minute time period, according to the method of Robbins and Marcus (8), twice a day for three days. Yields were 15 to 60 million cells per 50 flasks, depending upon the confluency of the cells in the flasks being shaken. To obtain cells at G_1 and S, T-150 flasks were each inoculated with 10 million mitotic cells and grown for 4 hr (G_1 phase) and 17 hr (S phase). The cells were washed twice with isotonic saline buffered with 10 mM Tris·HCl, pH 7.4, scraped into this buffer and washed further by centrifugation. Yields of the G_1 and S phase cells were 80 to 90% of the inoculum. Positions in the cell cycle were determined from a previously standardized cell cycle. To obtain this curve, synchronously growing cultures were taken at hourly intervals and pulsed with tritiated thymidine. The acid insoluble radioactive incorporation was used to define the S phase (9).

Plasma Membrane Preparation: Plasma membranes were prepared by the method of Tu, et al. (10) with a few modifications. The supernate from the 200 x g centrifugation of the cell homogenate was called the cytosol fraction. The pellet from this centrifugation was suspended in only two mls of the buffered 45% sucrose solution. After sonification, the sucrose suspension was placed in a five ml cellulose nitrate tube, two mls of buffered 30% sucrose was layered on top and one ml of 20% sucrose overlayed. The gradients were centrifuged at 100,000 x g for three hours in a SW 50 rotor. The pellet was called the nuclear fraction. The material at the interface of the 45% and 30% sucrose gradients was the plasma membrane fraction. It was collected and washed by sedimentation as previously described (10).

Analytical Techniques: The plasma membranes were solubilized in 3% SDS-1% mercaptoethanol, and analyzed by 10% SDS polyacrylamide disc gel electrophoresis which were stained for protein with Coomassie Blue (11). Duplicate gels were stained histochemically to determine the position of alkaline phosphatase (12).

Alkaline phosphatase activity was determined using p-nitrophenylphosphate as the substrate (13). Protein was determined by the method of Lowry , et al. (14).

RESULTS AND DISCUSSION

Table 1 shows that the specific activity of alkaline phosphatase in the plasma membrane of mitotic cells is over three fold ($P<0.05$) that found in plasma membranes of G_1 and S phase cells. This dramatic increase was seen in

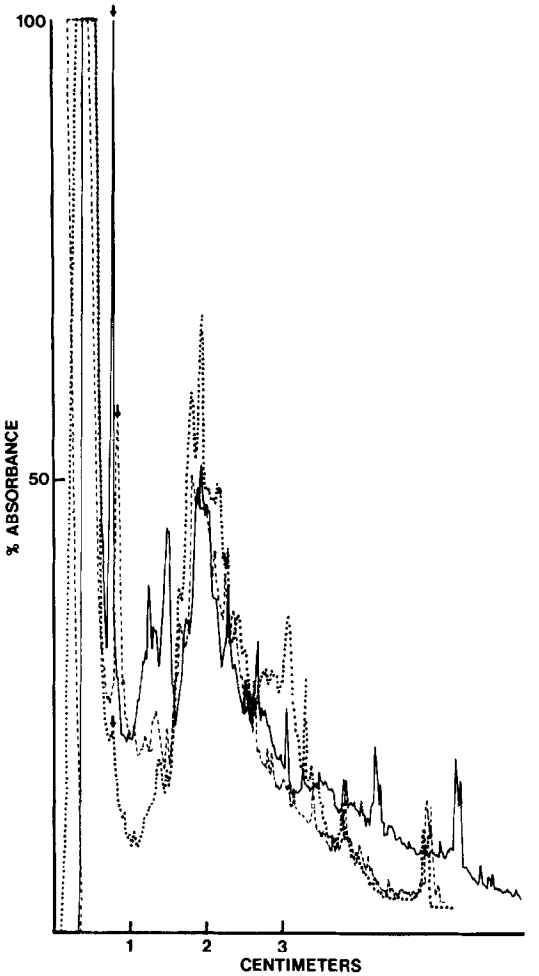

FIGURE 1

DENSITOMETER SCANS OF SDS POLYACRYLAMIDE DISC GELS

Each gel was loaded with 0.01 mg of plasma membrane protein from either M (- - -), G_1 (·····), or S (——) phase cells which had been solubilized in 3% SDS - 1% mercaptoethanol. Electrophoresis was performed at 8 ma/gel for eight hours and the gels stained and scanned on a Gilford 6050 recording spectrophotometer set at 540 nm. Arrows indicate alkaline phosphatase position.

several separate cell cycle experiments. Plasma membranes from G_1 and S phase cells are enriched approximately two fold in alkaline phosphatase compared to the respective cytosol and nuclear fractions (2.2 versus 0.89 or 1.2), whereas plasma membrane from mitotic cells contain seven times the specific activity of that in the cytosol and nuclear fractions (7.2 versus 0.9 or 1.5).

TABLE 2

SPECIFIC ACTIVITY OF ALKALINE PHOSPHATASE CALCULATED ON AMOUNT OF ALKALINE PHOSPHATASE PROTEIN IN GEL SCAN

Position in Cell Cycle	Area Under Alkaline Phosphatase Peak In Gel Scan*	Specific Activity of Plasma Membrane Alkaline Phosphatase	Specific Activity Alkaline Phosphatase/Area Under Alkaline Phosphatase Peak in Gel Scan
M	2.0	7.2	2.4
S	11.0	2.3	0.2
G_1	1.0	2.2	2.2

* Normalized by setting area under alkaline phosphatase peak in the G_1 phase gel scan equal to one

There is no significant difference in alkaline phosphatase activity among the M, G_1, and S cytosol fractions, or among the nuclear fractions. Furthermore, there is no significant difference ($P<0.2$) in alkaline phosphatase activity when the cytosol fractions from M, G_1, and S are compared to the corresponding nuclear fractions.

The next question was whether the increase in mitotic plasma membrane alkaline phosphatase activity was due to an increased amount of alkaline phosphatase protein in the membrane or whether it was due to modification of this protein near M, resulting in increased intrinsic activity. SDS polyacrylamide disc gel electrophoresis scanning patterns provided the answer. Figure 1 shows scanning patterns of 10% gels loaded with equal amounts of SDS solubilized plasma membrane proteins from either M, G_1, or S phase cells. Two cells for each phase were analyzed concurrently. One from each set was stained histochemically to locate the alkaline phosphatase activity, whereas the duplicate gel was stained with Coomassie Blue for protein. From the gel patterns, it can be seen that there was much more alkaline phosphatase protein in the S phase plasma membrane than in the G_1 and M phase plasma membranes (Fig. 1). The area under each alkaline phosphatase peak was calculated. Table 2 shows that the S phase plasma membrane contained 5.5 times as much alkaline phosphatase protein as the M phase (11/2) and 11 times that in the G_1 phase plasma membrane (11/1). The M phase plasma membrane contained two times as much

alkaline phosphatase protein as the G_1 phase membrane. Calculations of alkaline phosphatase specific activity per amount of alkaline phosphatase protein shows this activity to be 12 times higher in M than it is in S (2.4/0.2), but is the same for M and G_1 plasma membranes. These ratios indicate that the enhanced specific activity found in membranes from M phase cells is not due to an increase in enzyme protein, but rather to greater intrinsic enzyme activity. The increased amounts of enzyme protein in plasma membrane from S phase cells may reflect greater synthesis of this protein in S relative to other phases of the cell cycle.

The highest alkaline phosphatase activity in intestinal tissue is known to be located in the microvilli of the brush borders (15). HeLa cells have been shown to have more microvilli present during mitosis than at other points in the cell cycle (16). We found the mitotic plasma membrane to have the highest alkaline phosphatase activity. Therefore, the HeLa plasma membrane alkaline phosphatase may be located in the microvilli. Thus, microvilli may not be just a reservoir for plasma membrane, has been suggested (17), but may include modified membrane enzymes.

HeLa alkaline phosphatase is not the only protein that varies during the cell cycle. Figure 1 shows that plasma membrane from each phase of the cell cycle has a unique gel pattern. This altered pattern of membrane proteins is also seen when the same samples are analyzed on five per cent gels. These different patterns indicate that it may not be valid to compare plasma membrane proteins from different cell lines (i.e., nontransformed and transformed) without taking into consideration the difference in growth kinetics. In other words, if one cell line contained a higher percentage of its cells in one phase of the cell cycle as compared to the other, then some of the differences seen on polyacrylamide disc gel elctrophoresis could be due to cell cycle differences.

Although no significant variations in alkaline phosphatase activity are detectable in total homogenates at any point in the cell cycle (18), using the

same synchronous selection techniques a significant increase is found in isolated plasma membranes of mitotic cells. These results show the necessity for examining subcellular localizations when comparing various membrane biochemical parameters throughout the cell cycle. Moreover, it appears that high specific activity HeLa alkaline phosphatase can be used as a marker for the mitotic plasma membranes.

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