THE RELATIONSHIP BETWEEN SURFACE PROTEASE ACTIVITY AND THE RATE OF CELL PROLIFERATION IN NORMAL AND TRANSFORMED CELLS

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SUMMARY

Surface protease activity and secreted protease activity has been determined on several cell lines utilizing ^{3}H-acetyl casein as substrate. When normal rabbit aortic smooth muscle cells and fibroblasts stopped proliferating at high cell density a decrease in surface protease activity was observed. No decrease in surface protease activity or in the rate of cell proliferation was observed in either transformed melanoma or epidermal cells. The decrease of surface protease activity was related to a decrease in cell proliferation.

INTRODUCTION

Surface proteolytic enzyme activity has been demonstrated in normal and transformed cell lines (1-4). A specific serine proteinase, plasminogen activator, is associated with the plasma membrane fraction of normal and transformed cells (2,4). Plasminogen activator acting through plasmin appears to be responsible for certain morphological changes in transformed cells (5-10). Although the cellular function of the endogeneous surface proteases is not clearly understood, alterations in the growth rate, agglutinability and morphology of cultured cells can be produced utilizing either exogeneous protease(s) (11-16) or specific protease inhibitors (13,17,18,19).

We have demonstrated non plasminogen activator surface protease activity in several normal and transformed cell lines (3). There is a relationship between non plasminogen activator surface protease activity and doubling time.

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Cells with long doubling times have small amounts of surface protease activity while cells with rapid doubling times have large amounts of surface protease activity. The maximal surface protease activity was observed just before or during mitosis in several synchronized cell lines. In the present investigation surface protease activity was investigated in growth inhibited normal rabbit aortic fibroblasts and smooth muscle cells and in transformed human melanoma (HM-34) cells and mouse epidermal (UT-1) cells which were not growth inhibited.

MATERIALS AND METHODS

Rabbit aortic fibroblasts and smooth muscle cells were obtained from rabbit abdominal aorta. The human melanoma cells (HM-34) are from lines grown from melanoma tissue and were obtained from Dr. J.C. Bystryn, New York University, N.Y.C. The transformed epidermal (UT-1) cells were obtained from Dr. S. Yuspa, NCI, Bethesda, Md. The transformed epidermal cell line (UT-1) was produced by treating pregnant Balb/c mice in utero with urethane. Epidermal cells from the newborn mice were treated in culture with tetradecylphorbal acetate, (Yuspa, unpublished results).

Rabbit smooth muscle cells (passage 20-25) and fibroblasts (passage 12-15) are grown in medium 199 containing unmodified Earl's salts with 10% fetal calf serum according to the method described by Ross (20). Human melanoma cells and transformed mouse epidermal cells are grown in Dulbecco's modified Eagles media containing 10% newborn calf serum. In experiments in which protease activity was determined at 24 hour intervals the media on each plate was changed at 24 hour intervals otherwise the media was changed at 48 hour intervals. The cells are counted on duplicate plates employing a hemacytometer (Bright-line, Spencer, American Optical Corporation, Buffalo, N.Y.). After each experiment was completed the cells was examined by Eosin-Y exclusion.

Surface protease was determined according to method described by Hatcher et al (3). The [^{3}H]-labelled casein had a specific activity of 3,840 cpm per pmol assuming a molecular mass of 121,700 daltons. The cell cultures grown in Falcon petri dishes are washed 6X with Dulbecco's modified Eagles media without serum and incubated for 1.5 h at 37°C under 7% CO_2 in room air. The media is removed and 300 µl [^{3}H]-labelled casein containing 20 µg casein and 2 x 10^6 cpm previously dialyzed against serum free media (600 µl) containing EACA in that the final concentration of EACA in the reacting mixture was 10 mg/ml. The plates are then incubated for 180 min at 37°C under 7% CO_2 in room air. Following termination of the reaction by the addition of unlabelled casein (100 µl, 3% w/v in 1 M KCl) to an aliquot of the media (100 µl) followed by chilled 6% TCA (200 µl) the mixture is allowed to remain overnight in an ice slurry. The samples are then centrifuged at 15,000 rpm for 30 min and 200 µl of supernatant is added to 10 ml of Riafluor (New England Nuclear) and then the radioactivity determined. Surface protease activities are reported only when no secreted activity was detected since the surface protease assay also measure secreted activity.

Secreted protease activity was determined as previously described (3). Replicate plates containing serum free media (900 µl) are incubated for 180 min at 37°C under 7% CO_2 in room air. The media is removed, centrifuged at 15,000

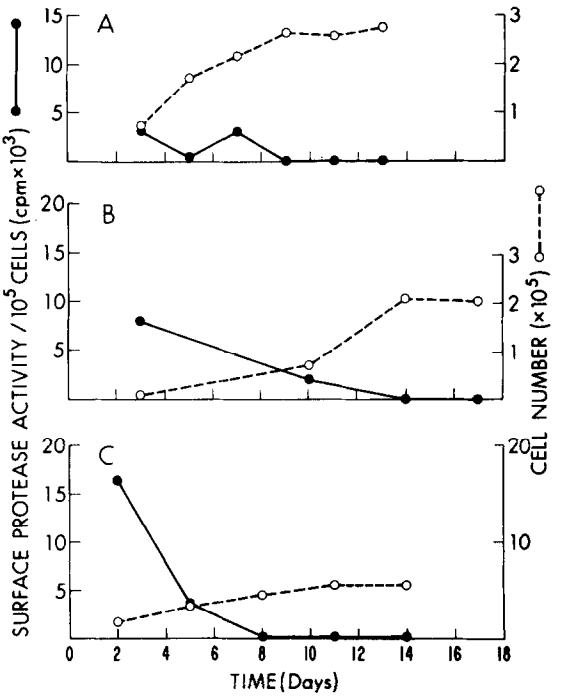

Figure 1.

Surface protease activity of: A. Rabbit aortic smooth muscle cells dispersed in trypsin (0.25% w/v)-EDTA (0.5 mM) (Grand Island Biological Co., Grand Island, N.Y.). B. Rabbit aortic smooth muscle cells dispersed with sterilized glass roller beads (3mm, Thomas Philadelphia, Pa). C. Rabbit aortic fibroblasts dispersed in trypsin (0.25% w/v)-EDTA (0.5 mM). Time 0 refers to the day the cells were dispersed and subcultured. Each point on the graph represents means values of triplicate counts of duplicate determinations on separate plates.

cpm for 15 min and an aliquot (100 µl) incubated for 120 min at 37°C with [^{3}H]-labelled casein (40 µl, 20 µg, 2 x 10^6 cpm). The reaction mixture contained ε-aminocaproic acid (EACA) at a concentration of 10mg/ml. The reaction is terminated with unlabelled casein (100 µl, 3% w/v in 1 M KCl) and 6% TCA (200 µl). The mixture is allowed to remain overnight in an ice slurry after which the samples are centrifuged at 15,000 rpm for 30 min and 200 µl of supernatant is added to 10 ml of Riafluor (New England Nuclear) and then the radioactivity measured.

RESULTS AND DISCUSSION

In Figure 1A surface protease activity of rabbit smooth muscle cells dispersed in trypsin-EDTA and subcultured are shown. At 0 and 1 day after subculturing, secreted protease activity was detected. The amount of combined

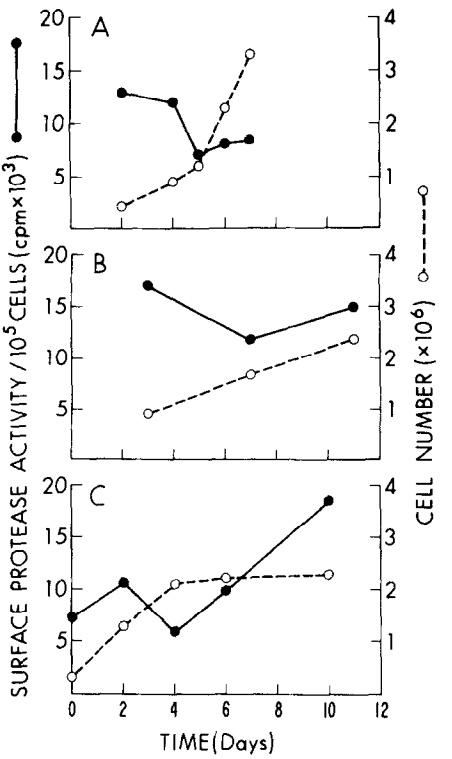

Figure 2.

Surface protease activity of human melanoma (HM-34) cells dispersed with A. Trypsin (0.25% w/v)-EDTA (0.5 mM). B. Sterilized glass roller beads. C. EDTA (1 mM). Day 0 refers to the time the cells were dispersed and subcultured. Each point in the graph represents means values of triplicate counts of duplicate determinations on separate plates.

secreted and surface protease activity was greater than 30,000 cpm/10^5 cells. (results not shown). Surface protease activity is only reported when no secreted activity was detected since the ^{3}H-acetyl casein assay for surface protease activity does not differentiate between surface and secreted protease activity. The surface protease activity of rapidly growing smooth muscle cells (3,5 and 7 days) was between 1000-3000 cpm/10^5 cells. A complete loss of surface protease activity was observed on days 9, 11 and 13. No increase in cell number was observed on days 9, 11 and 13. In order to insure that the observed proteolytic activity was not due to the effect of trypsin and EDTA, the cells were mechanically dispersed with sterilized glass beads and subcultured

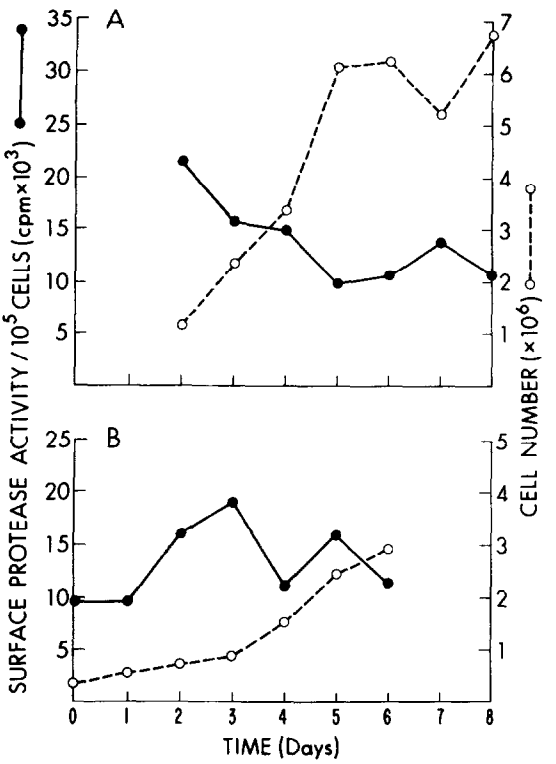

Figure 3.

Surface protease activity of mouse (balb/c) transformed epidermal cells (UT-1's) dispersed with A. Trypsin (0.25% w/v)-EDTA (0.5 mM). B. EDTA (1 mM). The cells were dispersed and subcultured on day 0. The points on the graph represent mean values of triplicate counts of duplicate determination on separate plates.

(Figure 1B). On day 3 and 10 when no secreted protease was detected, surface protease levels of 8000 cpm/10^5 cells and 2000 cpm/10^5 cell were found. On day 14 when the cells stopped multiplying, there was a total loss of surface protease activity. Surface protease activity of rabbit aortic fibroblasts dispersed with trypsin-EDTA and subcultured are presented in Figure 1C. The fibroblasts secreted large amounts of protease up to and including day 1 after subculturing. At day 8 a loss of surface protease activity was found and there was no subsequent increase in the number of fibroblasts.

Surface protease activity was also measured on human melanoma (HM-34)

cells and transformed epidermal (UT-1) cells which are not growth inhibited. In all experiments the transformed cells at high densities piled up and came off the plates. In Fig 2A and Fig 2B surface protease activity of human melanoma (HM-34) cells are presented at days 2-7 and days 3-11 when the cells did not secrete protease activity. No secreted protease activity was detected from day 0 to day 10 in HM-34 cells dispersed in EDTA (Fig 2C). The HM-34 cells dispersed in EDTA came off the plates at high cell density although rapid proliferation continued (Fig. 2C). Although a large fluctuation in surface protease activity/10^5 cells was observed no loss of surface protease was detected in the rapidly growing HM-34 cells. Surface protease activity of transformed epidermal (UT-1) cells dispersed with trypsin-EDTA (Figure 3A) and EDTA (Figure 3B) are shown. The UT-1 cells dispersed in EDTA did not secrete protease activity. Again no loss of surface protease activity was observed. Similar results were also obtained with fibroblasts dispersed with glass roller beads.

In the present investigation we have demonstrated a loss of surface protease activity when the cells stopped proliferating in rabbit aortic fibroblasts and smooth muscle cells. The loss of surface protease was not observed in rapid growing human HM-34 cells and mouse UT-1 cells. Bosmann et al (21) have observed that neutral protease activity in homogenates of rapidly dividing early passage WI-38 cells is not detectable in non dividing late passage WI-38 cells. They suggest that the neutral protease activity is present at the cell surface and that the loss of this protease activity may be responsible for the inability of late passage WI-38 cells to divide. It is possible this neutral protease activity is similar to our cell surface protease activity. In our study the loss of surface protease activity was related to a decrease in cell proliferation and was not associated with a terminal cellular event.

Noonan (22) has suggested that exogeneous proteases may affect the growth rate of cultured cells by altering the plasma membrane to increase the

availability of nutrients to cells. This is in agreement with Holley's hypothesis (23) that the initiation of the cell cycle is due to increase of nutrients or growth factors across the cell membrane. In the cell these events may be mediated through cell surface proteases in the plasma membrane.

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