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MOLECULAR CHANGES IN CELL SURFACE MEMBRANES RESULTING FROM TRYPSINIZATION OF SARCOMA 180 TUMOR CELLS*

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SUMMARY

Sarcoma-180 tumor cells in culture or grown as an ascites form in the CD-1 mouse have been subjected to mild trypsinization procedures in order to study morphological and molecular changes resulting from proteolysis. The cells attached to a substratum become rounded within 20 min and most undergo cell division, but they do not detach from the substratum. Removal of trypsin permits the cells to go back to their original spindle shape over an 8–20 h period.

Surface membranes were isolated from trypsinized ascites and cultured cells and subjected to dodecyl sulfate-acrylamide gel electrophoresis. Both cell types showed the same two kinds of changes in electrophoretic patterns. First, there was a loss of glycoproteins from both cell types, even though they show different complements of cell surface glycoproteins. Second, there is a loss of high molecular weight polypeptides, which have previously been suggested to play a role in membrane stabilization and cell shape. These results further implicate these polypeptides in the control of cell morphology and offer circumstantial evidence for transmembrane interactions of surface glycoproteins with the high molecular weight polypeptides as a factor in controlling cell morphology.

INTRODUCTION

Mild proteolysis of cultured fibroblasts causes changes that are similar, but not necessarily identical, to those which occur during cell transformation [1]. Included among these are alterations in cell morphology [2], cell agglutinability by lectins [3], distribution of surface lectin receptors [4] and growth rate [5]. Lacto-

Abbreviations: 199, medium 199 minus calf serum; S-180, sarcoma 180 tumor grown in cell culture; SA-180, sarcoma 180 tumor grown in vivo as ascites form; PMSF, phenylmethanesulfonyl fluoride; Me_2SO, dimethylsulfoxide; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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peroxidase labeling indicates that some fibroblast lines contain a surface protein which is absent in their virally transformed counterparts and which is extremely sensitive to proteolysis [6]. Transformed cells secrete a plasminogen-activating factor [7] as well as other proteolytic activities [8]. Although plasmin does not appear to be responsible for maintaining the transformed state [9], it is not known what role proteases may play in transformation.

The effects of proteases on cellular morphology are of particular interest. If morphology is controlled by an internal cytoskeleton, proteases must be able to cross the plasma membrane to attack cytoskeletal elements or there must be a transmembrane system involved in morphology control in order to explain protease effects on morphology. In the erythrocyte, cell shape appears to be maintained by a set of microfibrillar elements which contain spectrin [10] and possibly an erythrocyte actin [11]. A transmembrane connection has been shown between spectrin and the erythrocyte surface glycoprotein [12], but the interaction does not appear tc be strong enough to cause red cell shape changes by proteolytic degradation of the glycoproteins [13].

In seeking to understand the membrane elements important to cell morphology in eukaryotic cells, we have sought spectrin-like proteins in more complex cells. Recently we have described a set of high molecular weight membrane-associated polypeptides in sarcoma 180 tumor cells, which may play a role in membrane stabilization and cell shape [14]. These polypeptides are found associated with intact cell envelopes or large membrane fragments isolated after treating the cells with membrane "stabilizing" agents, but they are not found in membrane vesicles isolated in the absence of stabilizing procedures [14]. If the membrane envelopes are treated with EDTA, the envelopes collapse to vesicles and two of the high molecular weight polypeptides are extracted along with a polypeptide of molecular weight 45 000 (unpublished observations). These results parallel the behavior of erythrocyte ghosts, from which spectrin and erythrocyte actin can be extracted with concomitant vesicularization. It has been suggested that these proteins play important roles in erythrocyte shape [11]. We now report changes in the ascites high molecular weight membrane polypeptides and surface glycoproteins resulting from a mild trypsinization procedure which causes morphological changes in the cells. These results indicate the possible importance of the high molecular weight polypeptides to cell shape and suggest that they may be involved in transmembrane interactions similar to those postulated for spectrin of the erythrocyte membrane [10].

EXPERIMENTAL PROCEDURES

Materials

Medium 199 was purchased from Grand Island Biological Co. and serum from Microbiological Associates. S-180 cell cultures were purchased from the American Type Culture Collection Cell Repository (CCRF-S180 II). Trypsin (Type I, bovine pancreas) was obtained from Sigma Chemical.

Time-lapse cinematography of the effect of trypsin on the S-180 cell

Cinematography studies were carried out on a Dvorak-Stotler Controlled

Environment Culture System. S-180 cells suspended in 199+10 % calf serum were inoculated into the chamber and incubated (inverted) in a CO_2 gas phase incubator at 37 °C for 4 h to allow the cells to attach to the coverslip. The chamber was then placed onto the microscope stage and perfused with 199+10 % calf serum at 1 ml/h. The chamber temperature was maintained at 37 °C by a Sage air curtain.

Photography was performed using phase contrast optics and a 16 mm time-lapse system. Sequences were filmed at 15 frames/min with a 0.75 s exposure using Kodak Tri-X No. 7278 reversal film.

Cells were perfused for 12 h prior to experimentation to allow flattening and characteristic spindle morphology to occur. The culture was then filmed for at least 1 h to record its characteristics as a control. Trypsin (10 μg/ml in 199 minus calf serum) was added by flushing the chamber to insure uniform trypsin exposure. Filming was continued throughout the process and 15 min after trypsin addition the chamber was flushed with normal medium (199+10 % calf serum) and returned to perfusion at 1 ml/h. The filming was continued to record changes in cell morphology. Control sequences were run in which only 199 minus calf serum without trypsin was used in place of trypsin. These sequences showed no change in cell morphology either during or following the 15 min 199 minus calf serum exposure. Experiments were also run with trypsin in 199 plus 10 % calf serum and showed essentially equivalent results.

Trypsinization

Five days after implantation, SA-180 cells were aseptically removed from the mouse peritoneal cavity, washed three times in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffered saline [15] and once in 199 at 4 °C. After resuspension in 199 to 10^4 cells/ml and warming to 37 °C, the cell suspensions were treated with trypsin at concentrations of 0–5 μg/ml at 37 °C. As a control for endogenous proteases, samples were also kept on ice without trypsin to compare with untrypsinized samples incubated at 37 °C. After 15 min the action of trypsin was stopped either by adding a 20-fold excess of phenylmethanesulfonyl fluoride (PMSF) in dimethylsulfoxide (Me_2SO) and allowing the cells to incubate 30 min at room temperature [13] or by adding a 100-fold excess of soybean trypsin inhibitor at 4 °C and incubating for 30 min. Control cells were identically treated except for the presence of trypsin. Cells were washed four times with HEPES-buffered saline, and surface membranes were isolated by the zinc two-phase method [14].

S-180 cells were grown in milk dilution bottles. Just prior to reaching confluency, the cells were harvested with a rubber policeman and washed three times with HEPES-buffered saline at 4 °C. The cells were washed once in 199, suspended in 199 at a concentration of 10^4 cells/ml and trypsinized as described above. Membranes were prepared by the zinc two-phase method.

Electrophoresis

Freshly prepared membrane samples were solubilized immediately by boiling 5 min in 4 % dodecylsulfate to avoid degradation from storage and solubilization [16]. Samples were subjected to electrophoresis on 5 % acrylamide gels for 5 h at 8 mA/gel [13]. Gels were stained with Coomassie blue or periodate-Schiff reagent [13, 14].

RESULTS

The effect of trypsin on the sarcoma-180 plasma membrane

Mild trypsinization of the cultured S-180 causes pronounced changes in cel-

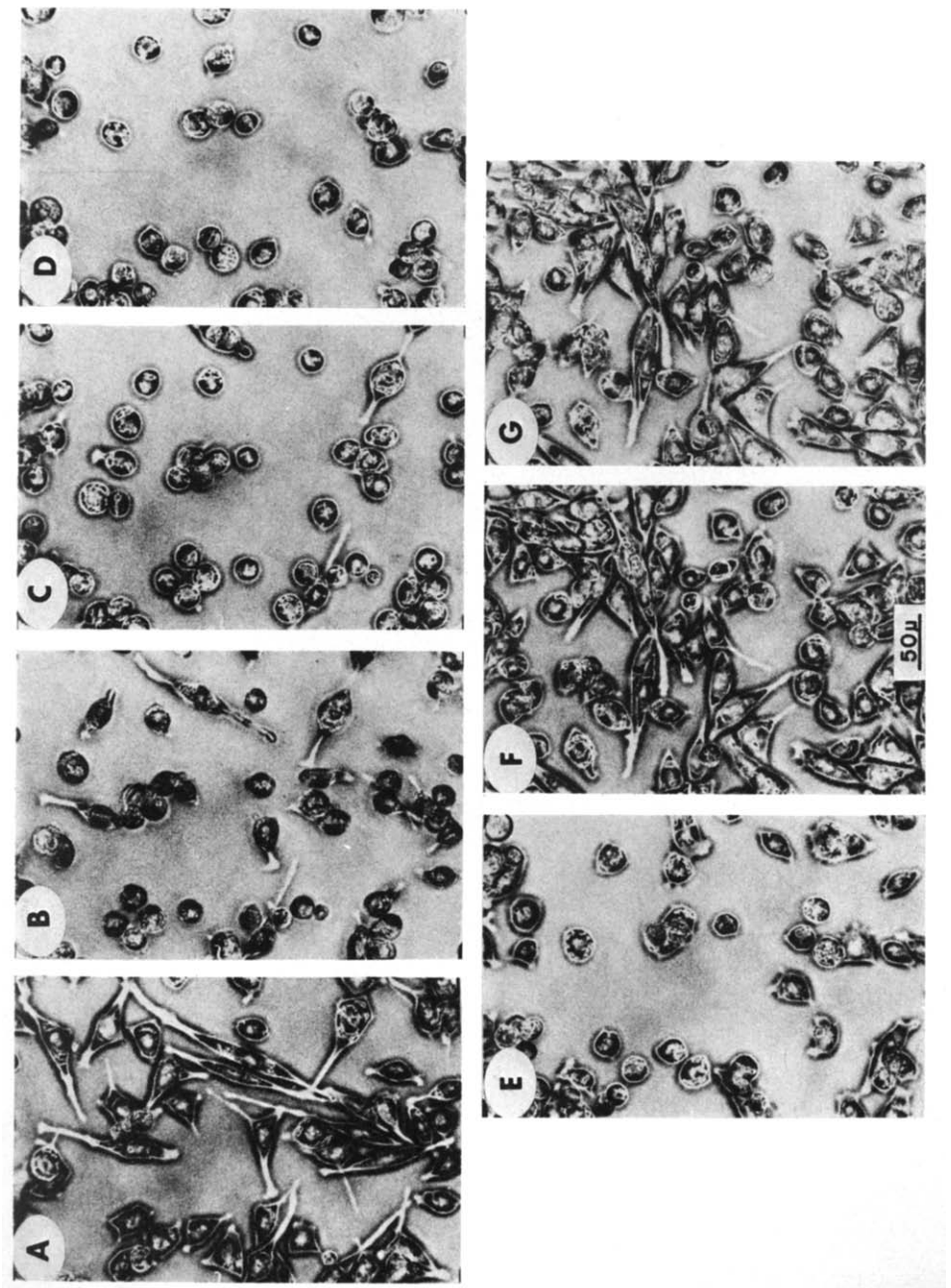

Fig. 1. Morphological effects of trypsinization on S-180 tumor cells and its reversal. The sequence of events shown in Frames A–G is explained in the text.

lular morphology as shown by phase contrast time-lapse cinematography. Fig. 1 shows the results of trypsin treatment on S-180 cells attached to a glass substrate. The first picture (Frame A) was taken 4 min after trypsin addition and shows a culture in which no morphological changes have yet occurred. Frame B, taken 12,5 min after trypsin addition, shows a number of cells that have begun to withdraw their appendages and become rounded. At 15 min the trypsin is removed and the cells are perfused with the M-199+calf serum. Frame C shows the cells 16.3 min after trypsin addition and Frame D, taken 20 min after trypsin addition, shows that all the cells are rounded with only minute projections, very similar to cells undergoing mitosis.

Frame E, taken 8 h after trypsin treatment indicates some flattening of the cells has begun to take place. This flattening and spindle formation continues and in Frames F and G, 20 and 24 h after trypsin treatment, respectively, the cells have regained the characteristic fibroblast morphology observed in Frame A. Many cells have undergone at least one division. Only two or three of the cells in the original field became detached from the glass and swept away by the perfusion medium. Most of the cells did undergo subsequent division following trypsin treatment indicating that the tryptic action, while able to cause a dramatic change in cell morphology, does not cause loss of cell viability.

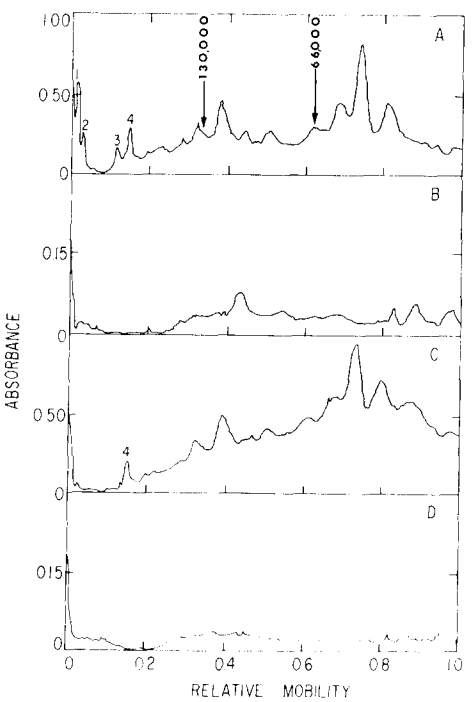

Fig. 2. Polypeptide and glycoprotein patterns of surface membranes from untreated and trypsinized SA-180 cells. A and B, Coomassie blue and periodate-Schiff patterns, respectively, from control samples; C and D, Coomassie blue and periodate-Schiff patterns from trypsinized samples. Numbered bands denote areas of most significant changes. Approximate molecular weights of the numbered species on the Coomassie blue profile are: 1, ~ 300 000; 2, 300 000; 3, 240 000; 4, 220 000.

Because of the pronounced effects of trypsin on cell morphology as demonstrated with these cells and in other studies [2], it was of interest to determine what membrane changes occur at the molecular level as a result of trypsinization. Surface membranes were prepared from control and trypsinized cells by the two-phase isolation procedure [14] and subjected to acrylamide gel electrophoresis in sodium dodecyl sulfate. Polypeptide and glycoprotein patterns of membranes from trypsinized (5 μg/ml) and untreated SA-180 cells are shown in Fig. 2. Trypsinization causes an almost complete elimination of the major glycoprotein bands and a dramatic loss of a group of high molecular weight polypeptides (Bands 1–4 of scan B) which are different from the glycoproteins. There are no other significant changes in the patterns except for an increase in material in the lower regions of the gels, probably arising from degradation products. The high molecular weight polypeptides lost during trypsin treatment correspond to those which we have suggested are involved in "stabilization" of the membrane as sheets during homogenization with agents such as Zn^{2+}. These polypeptides are present in the "stabilized" membrane sheets or envelopes but not in vesicular membrane preparations [14].

Similar results were obtained with the S-180 cells grown in culture, which show a different complement of surface glycoproteins [17]. Both glycoproteins and high molecular weight polypeptides have been essentially lost from the electrophoresis patterns of the membranes of the trypsinized cells (Fig. 3). Comparative electrophoretic analyses indicate that bands 3 and 4 from the Coomassie blue scans coin-

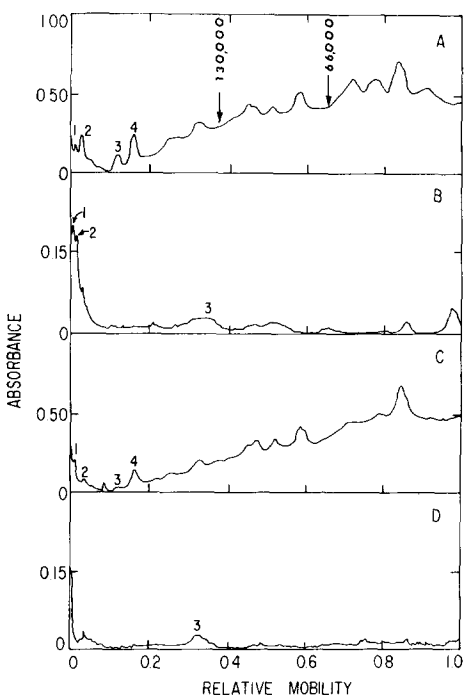

Fig. 3. Polypeptide and glycoprotein patterns of surface membranes from untreated and trypsinized S-180 cells. Individual patterns are lettered and numbered as in Fig. 2.

cide almost exactly with the spectrin bands of erythrocyte membranes. It is interesting to note that there is not simultaneous degradation of these two bands. Somewhat similar behavior is exhibited by spectrin [13]. The slower migrating bands, particularly band 1, may represent aggregated material.

DISCUSSION

Mild trypsinization of sarcoma 180 tumor cells (S-180 and SA-180) causes the loss of two different types of component from their surface membrane electrophoretic profiles. One of these is the glycoproteins of the surface membranes, and the other is a group of high molecular weight polypeptides associated with surface membranes of cells treated with zinc [14] or other membrane "stabilizing" agents [18]. Proteolytic cleavage of glycoproteins at the cell surfaces was expected, since this behavior has been demonstrated for a number of mammalian cell types by either release of glycopeptides [19] or loss of the glycoproteins from electrophoretic profiles [6]. The loss of the high molecular weight polypeptides was not expected. There are three alternatives which we consider most likely to explain this latter result. (1) The polypeptides are present at the exterior surface of the cell and cleaved by trypsin acting on the cell surface. (2) The polypeptides are associated with the interior surface of the membrane and cleaved by trypsin, which passes into the cell. (3) The polypeptides are associated with the interior surface of the membrane and are released from this association as a result of membrane structural alterations that occur as a result of proteolysis of cell surface components. Our current results cannot distinguish among these alternatives. Lactoperoxidase labeling did not aid in localizing the high molecular weight polypeptides, since they were not labeled with either intact cells or isolated, fragmented membranes [14, 18]. Their apparent ability to stabilize the membranes might suggest a location at the inner membrane surface, as with the erythrocyte membrane structural protein spectrin [20]. However, another structural protein, myosin, has been found on cell surfaces [21]. The possibility of specific cleavage of the high molecular weight components by trypsin inside the cell appears less likely. If trypsin does enter the cells, it would have to cleave the high molecular weight polypeptides specifically to explain our results. It does not exhibit this specificity with isolated membranes [18].

The third hypothesis is particularly attractive because it relates elements inside the cell, which may be involved in maintaining membrane shape, to events which occur at the cell surface. Such a relationship is presumably required to explain effects of agents such as adenylate cyclase effectors on cell morphology [22]. Similar interactions have been proposed in the erythrocyte membrane between the surface glycoproteins and spectrin [12, 23], which apparently plays a role in cell shape and membrane stabilization [24]. There are noteworthy similarities between the properties of the polypeptides studied in this report and spectrin [18]. Investigations of receptor mobility in membranes of complex cells have suggested the interaction of surface receptors with submembrane cytoskeletal elements [10]. It is not necessary that these interactions be direct, as has been proposed with the erythrocyte. Instead, they could involve cooperative effects such as have been observed for hormone-membrane interactions [25]. Since the strength and stability of the interactions may vary with the cell system and membrane isolation procedure, the loss of these polypeptides with

trypsinization may not necessarily be observed with other cell types or other isolation methods.

Although morphological changes that occur with trypsinization are very rapid, occurring in less than a minute at trypsin concentrations normally used in cell culture [2], they may not be the first events occurring after trypsinization. The cause-and-effect relationships resulting from trypsin treatment are still obscure, and the protein changes observed in this study may be a secondary effect of an earlier membrane alteration. Further characterization of the trypsinization process and of these polypeptides will be necessary to relate these observations to other cellular changes that occur with proteolysis.

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