Biochimica et Biophysica Acta, 413 (1975) 453–458

BBA 77138

CELL CYCLE DEPENDENT EXPOSURE OF A HIGH MOLECULAR WEIGHT PROTEIN ON THE SURFACE OF MOUSE L CELLS

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(Received May 23rd, 1975)

SUMMARY

The non-penetrating lactoperoxidase iodination probe has been employed in conjunction with synchronously dividing populations of mouse L cells to identify a high molecular weight protein which is preferentially exposed on the L cell surface during G1 phase of the cell cycle. Progression of cells from G1 to S is accompanied by a marked decrease in the availability of this structure, called band 1, to lactoperoxidase-catalyzed iodination and it remains unavailable until cells re-enter G1. It is suggested that the band 1 polypeptide may be functionally involved in the regulation of L cell growth.

INTRODUCTION

Recent studies in our laboratory and elsewhere have been devoted to identifying the protein and glycoprotein components exposed on the outer surface of mouse L cells [1–4]. Nonpenetrating biochemical probes such as the lactoperoxidase technique [5], the pyridoxal phosphate – NaB^3H_4 method [6] and the galactose oxidase – KB^3H_4 system [7] have been employed to introduce radioactive groups specifically into exposed structures and the labeled components have been identified by their mobility on sodium dodecyl sulfate-polyacrylamide gels. These studies have resulted in the identification of a high molecular weight protein (called band 1) which is exposed on the L cell surface and readily available to iodination with ^{125}I by the lactoperoxidase method. Similar structures have been observed on the surface of normal, but not transformed, hamster fibroblast [8, 9], chick embryo fibroblast [10, 11] and BALB/c 3T3 cells [12]. Here we show how the lactoperoxidase technique has been employed in conjunction with synchronously dividing populations of L cells to examine the effect of cell cycle processes on the exposure of band 1 on the cell surface. Our results indicate that the band 1 polypeptide is exposed on the cell surface only during G1 phase of the cell cycle and that progression of cells from G1 to S is associated with a striking decrease in the availability of band 1 to the lactoperoxidase probe. These findings are consistent with previous studies which have demonstrated a

decreased availability of structures similar to band 1 in mitotic NIL8 [14] and BHK-21 cells [15].

MATERIALS AND METHODS

Cell growth and synchronization

Suspension cultures of L-929 cells employed in these experiments were grown at 37 °C in medium 199 containing 20 % fetal calf serum, 0.06 % glutamine, 0.15 % $NaHCO_3$ and antibiotics (100 units/ml of penicillin and 100 μg/ml of streptomycin). Synchronously dividing suspension cultures were prepared from monolayer cultures of L cells in two different ways, by subculturing cells which had grown to stationary phase and by the double thymidine block method. In the first case monolayer cultures of L cells were allowed to grow to confluence (10^7 cells per 100 cm^2) on the surface of Falcon plastic culture plates in basal medium-Eagle containing 10 % calf serum, 0.14 g/l glutamine and antibiotics; cells were allowed to remain quiescent for 4 days after they had reached stationary phase. They were then removed from the plate by trypsinization (0.25 % trypsin for 10 min at 37 °C) washed in Dulbecco's phosphate buffered saline and transferred to a 250 ml spinner flask (Bellco Glass, Inc.) containing 200 ml medium 199 supplemented as described above. This cell suspension was incubated at 37 °C with constant stirring; the cell density was determined as a function of time using an improved Neubauer hemocytometer.

Synchronously dividing L cell populations produced by the double thymidine block method were prepared beginning with sparse, monolayer cultures grown in supplemented basal medium-Eagle containing 2 mM thymidine for 24 h. These cells were then shifted to basal medium-Eagle lacking thymidine for 15 h and, finally, re-exposed to thymidine-containing basal medium-Eagle for a further 24 h. A suspension culture was prepared from these cells and the number of cells/ml determined as a function of time using the methods described above.

In the case of cells synchronized by growth to stationary phase and in the case of cells synchronized by the double thymidine block method, the period of the cell cycle during which DNA was being synthesized (S phase) was determined by removing approx. 10^6 cells from the synchronized culture at various times and resuspending them in supplemented basal medium-Eagle containing 0.1 μCi/ml [^{3}H]-thymidine (6.6 Ci/mmol). After incubation of these cultures for 1 h at 37 °C, the cells were precipitated with 5 % trichloroacetic acid and collected on a Whatman GF/c glass fiber filter; the filters were dried and counted in a Packard liquid scintillation counter. Cells showing a high level of [^{3}H]thymidine incorporation by this test were assumed to be actively synthesizing DNA.

Lactoperoxidase-catalyzed iodination and sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Cells were iodinated by the lactoperoxidase method [5] using a modification of the procedure previously described [1]. Approx. 10^6 suspension cells were harvested by centrifugation, washed in phosphate-buffered saline and resuspended in 0.5 ml phosphate-buffered saline containing 10 μg lactoperoxidase (Sigma Chemical Co.) and 100 μCi $Na^{125}I$ (Amersham-Searle). The iodination reaction was initiated by the addition of 10 μl 0.1 % H_2O_2 and identical aliquots of H_2O_2 were added at 2-min

intervals over a period of 10 min incubation at room temperature. Incubation was continued for a further 10 min before cells were washed free of lactoperoxidase and excess $Na^{125}I$, dissolved in 0.1 ml 1 % sodium dodecyl sulfate, 1 % 2-mercaptoethanol and boiled for 2 min. The solubilized cells were then subjected to electrophoresis on a 7 % polyacrylamide gel containing 0.1 % sodium dodecyl sulfate [1]. Radioactive label was detected on the developed gel by slicing it laterally into 1-mm-thick discs and counting the discs in a toluene-based liquid medium.

RESULTS

L-929 cells to be labeled by the non-penetrating lactoperoxidase system were grown in suspension culture, washed twice in phosphate-buffered saline and then exposed to lactoperoxidase and H_2O_2 in the presence of $Na^{125}I$ as described above. Cell surface proteins labeled in this way were identified by their mobility on sodium dodecyl sulfate-polyacrylamide gels; labeled proteins were detected on the developed gels by slicing the gels laterally into 1-mm-thick discs and counting in a toluene-based liquid medium. When asynchronous cultures of L cells were labeled and analyzed by this procedure the results shown in Fig. 1 were obtained. Between 10 and 20 proteins having apparent molecular weights of 20 000–250 000 were found to be labeled with ^{125}I and separated from each other on the gel. This result with L cells grown and iodinated in suspension contrasts markedly to the situation with monolayer cultures of L cells where far fewer protein species are well labeled [1, 4]; the reason for this discrepancy is not clear at the present time. The most slowly migrating of the protein species labeled in suspension cells, which we call band 1 and whose position on the gel is indicated in Fig. 1, was ordinarily found to contain approximately 5 % of the total ^{125}I label applied to the gel and to migrate with an apparent molecular weight of 250 000. Similar structures have been observed in L cells by Poduslo et al. [4] and by Hubbard and Cohn [2]. Further studies of the band 1 polypeptide have served to confirm the view that it is exposed on the outer cell surface. For example, band 1 was

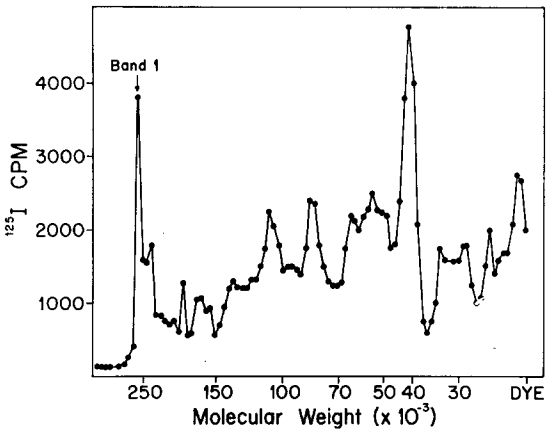

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of L cell surface proteins labeled with ^{125}I by the lactoperoxidase method.

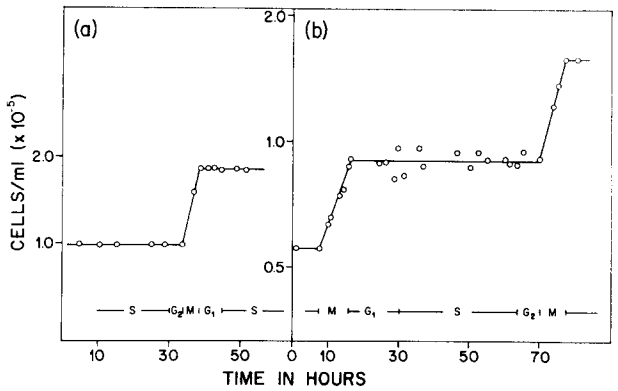

Fig. 2. Cell density in synchronously dividing L cell populations produced by growth to stationary phase (a) and by the double thymidine block method (b).

found to be digested when intact cells were treated briefly with trypsin (5 μg/ml trypsin for 10 min at 37 °C) and it was isolated with purified L cell plasma membranes prepared by the polystyrene latex bead technique [13].

The effect of cell cycle processes on the exposure of band 1 on the cell surface was examined by iodinating aliquots of synchronously dividing cell cultures at different stages of the cycle and comparing the amount of ^{125}I label incorporated into band 1. Synchronous cell populations were prepared for these experiments in two ways, by subculturing cells which had grown to stationary phase [16] and by the double thymidine block method [17]. In both cases cells were synchronized in monolayer culture and then transferred to 250 ml suspension cultures at an initial density of $0.5 \cdot 10^5$–$1 \cdot 10^5$ cells/ml. These cells were found to undergo one or more cycles of cell division in synchrony as shown in Fig. 2.

Approx. 10^6 cells were taken from these cultures at different times during the cell cycle, labeled with ^{125}I by the lactoperoxidase technique, solubilized and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described above. The amount of ^{125}I label in band 1 was determined from the developed gel and taken

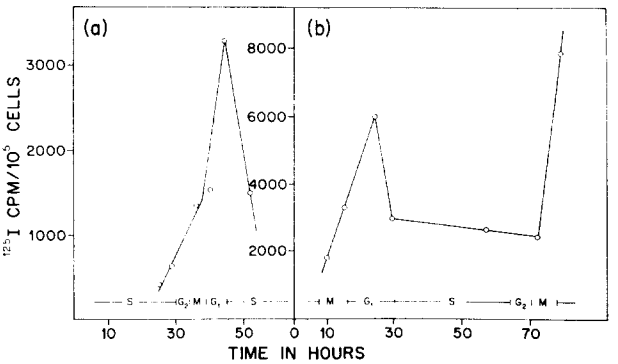

Fig. 3. Lactoperoxidase-catalyzed iodination of band 1 in synchronously dividing L cell cultures produced by growth to stationary phase (a) and by the double thymidine block method (b).

as an overall measure of the exposure of the band 1 polypeptide on the cell surface at the time of iodination. The results of this experiment, as shown in Fig. 3, were qualitatively similar in cells synchronized by growth to stationary phase and by the double thymidine block method. They indicate that exposure of band 1 on the cell surface was greatest during G1, although the total amount of ^{125}I label incorporated into cells was quite similar in all phases of the cell cycle. Progression of cells from G1 to S was accompanied by a marked decrease in the availability of band 1 to the lactoperoxidase probe and this low level of exposure was characteristic of cells throughout S and most of G2. Beginning in late G2 exposure of band 1 began to increase steadily before reaching a maximum again in G1. In contrast to the situation with band 1, other ^{125}I-labeled proteins did not show this type of preferential exposure on the cell surface during G1.

DISCUSSION

Our results show clearly that exposure of band 1 on the cell surface is linked to cell cycle processes. Like a similar structure in NIL8 cells [14], band 1 was found to be most accessible to lactoperoxidase-catalyzed iodination during G1 and quite significantly less accessible during other phases of the cell cycle. In this respect our observations are consistent with and complement other studies which have associated particular surface properties with cell cycle events [18–21]. In the case of band 1, however, appearance of this species on the cell surface during G1 raises the possibility that it may be functionally involved in progression of cells through the cell cycle rather than simply a secondary consequence of cell cycle processes. G1 is the phase of the cycle at which a cellular decision is reached regarding whether cells remain quiescent in G1 or become committed to DNA replication and cell division [22]. It is possible, in the case of band 1, that exposure of this structure on the cell surface is responsible for cells remaining in G1 and that conversion of band 1 to the unexposed state is itself the molecular signal which indicates that cells should begin to synthesize DNA and ultimately divide. This proposal is supported by the fact that a structure resembling band 1 was found to be significantly more exposed on the surface of density-inhibited (G1) than dividing hamster fibroblasts [14] and that a similar component was severely depleted or missing entirely from virus-transformed cells [8, 10–12]. Further evidence that band 1 is functionally and not just coincidentally involved in progression of L cells through the cell cycle will require that one understand the physical basis for why band 1 is accessible to the iodination probe during G1 and much less accessible at other times during the cell cycle. It would be of interest to know, for example, whether the band 1 protein is present only in G1 phase or whether it is present throughout the cell cycle, but accessible to lactoperoxidase only in G1. Experiments designed to make this distinction are currently in progress in our laboratory.

ACKNOWLEDGEMENTS

This work was supported by grants from the U. S. National Institutes of Health (HD-06390) and from the American Cancer Society (BC-183).

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