ACTIN POOLS IN EXTRACTS OF TERATOCARCINOMA CELLS, ASSAYED BY INHIBITION OF DNase I

Francis MARKEY and Luis SORIANO+

CEMO-group, Wallenberg Laboratory, University of Uppsala, Box 562, 751 22 Uppsala, Sweden and *+Unité de Chimie de Proteines, Institut Pasteur, Paris, France*

Received 14 December 1979

1. Introduction

Mouse embryonal carcinoma (EC) cells have been used as a model to study early embryonic development, since similarities between EC cells and uncommitted cells have been described [1–4]. EC cells will differentiate in vitro either spontaneously or in response to appropriate stimuli [5,6] to give rise to a variety of cell types in which derivatives of all 3 germ lines can often be identified. These differentiated derivatives generally lack the malignant properties of the original cells. Undifferentiated EC cells are rounded and only loosely attached to the substratum. Indirect immunofluorescence studies with antibodies against actin have shown that EC cell actin is not organized in stress fibres, but is distributed diffusely in the cytoplasm or concentrated in surface structures [7]. During differentiation, cells flatten, adhere more strongly to the substratum, and acquire characteristic arrangements of well-developed actin cables [7]. Differences between EC cells and differentiated derivatives have also been observed by measuring alkaline DNase activity. This enzyme activity is easily detected in EC cells when extracts are made at low ionic strength, whereas it is undetectable in differentiated cells under the same conditions [8]. Immunofluorescence studies on deoxyribonuclease in mouse teratocarcinoma cells using specific DNase-I IgG antibodies have shown that the staining patterns are quite different in EC cells (amorphous cytoplasmic fluorescence and absence of nuclear staining) and in differentiated derivatives (diffuse, bright granular and fibrillar fluorescence) [9].

The idea that reorganization of microfilament-containing structures in cells might involve a controlled exchange of actin between unpolymerized and filamentous forms was first proposed in [10] for the spreading of cells on solid substrates and has since been applied to a variety of cellular processes (reviewed [11]). Biochemical support for this hypothesis has been obtained from the stimulation of human platelets [12,13], using an actin assay based on differential inhibition of DNase I by monomeric and filamentous actin [14]. Here, we present measurements of actin pools, using the same DNase inhibition assay, in extracts of EC cells and of a fibroblast-like cell line derived from them. The results show that the proportion of actin in the unpolymerized pool from EC cells is about twice as high as that from the differentiated derivative, providing a biochemical marker for the reorganization of actin during differentiation.

It is characteristic of extracts of non-muscle cells that unpolymerized actin is present at a significantly higher concentration than that expected from the properties of purified actin [11]. The stability of this unpolymerized state can in several cases be attributed to combination of actin with the basic protein profilin, an inhibitor of actin polymerization [15–18]. Preliminary experiments demonstrate the presence of a profilin-like protein associated with unpolymerized actin in extracts of both EC cells and the differentiated derivative.

2. Experimental

The cell lines employed here and the culture conditions have been described in [19,20]. PCC3/A/1 and PCC4Aza have been used as EC cells. PCD3, a fibroblast-like cell line derived from PCC3/A/1 was used as the differentiated derivative.

Cell extracts were made by scraping the cells from

the substratum and then gently homogenizing in lysis buffer (150 mM NaCl, 5 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.2 mM ATP, 0.5% Triton X-100) by several strokes with a glass Potter homogeniser. About $1–2 \times 10^7$ cells were extracted in 0.5–0.7 ml lysis buffer. Aliquots were taken for measurement of DNase inhibitor activity before and after treatment with guanidine–HCl (final conc. 0.75 M in 0.5 M Na-acetate, 10 mM Tris–HCl (pH 7.5), 0.5 mM $CaCl_2$, 0.5 mM ATP), by the method in [14].

3. Results and discussion

Discrimination between unpolymerized and filamentous actin by the DNase inhibition assay in model systems is based on the different rates of interaction between DNase and the 2 forms of actin, so that under the conditions used inhibition by monomeric actin is essentially complete before appreciable inhibition from the filamentous form is detected. Unpolymerized actin is thus measured as the direct inhibitor activity of a sample; the total amount of actin is determined in the same way after depolymerization of filaments with guanidine–HCl [14]. Since the assay distinguishes 2 actin pools in cell extracts by the same criteria, these pools are referred to as unpolymerized and filamentous actin, even though the situation in cell extracts may not be so simple.

The results of DNase I inhibition measurements on extracts of PCC3 (undifferentiated) and PCD3 (fibroblast-like) cells are shown in fig.1. From the first measurements, 3 min after lysis, the unpolymerized actin pool in the PCC3 extract represents ~75% of the total actin, and this distribution is stable over 60 min at 0°C. Similar results were obtained with PCC4Aza cells (not illustrated). Corresponding figures for the extract of PCD3 cells are 35% shortly after lysis, rising to an essentially stable 45% of the total after ~40 min. In separate determinations, the difference in actin distribution between the cell types was maintained, although the absolute proportions of unpolymerized actin varied from 75–90% for PCC3 and from 32–50% for PCD3, and slight differences in the stability of the actin pools were seen. The difference in actin distribution was also maintained when extracts were made from frozen cells.

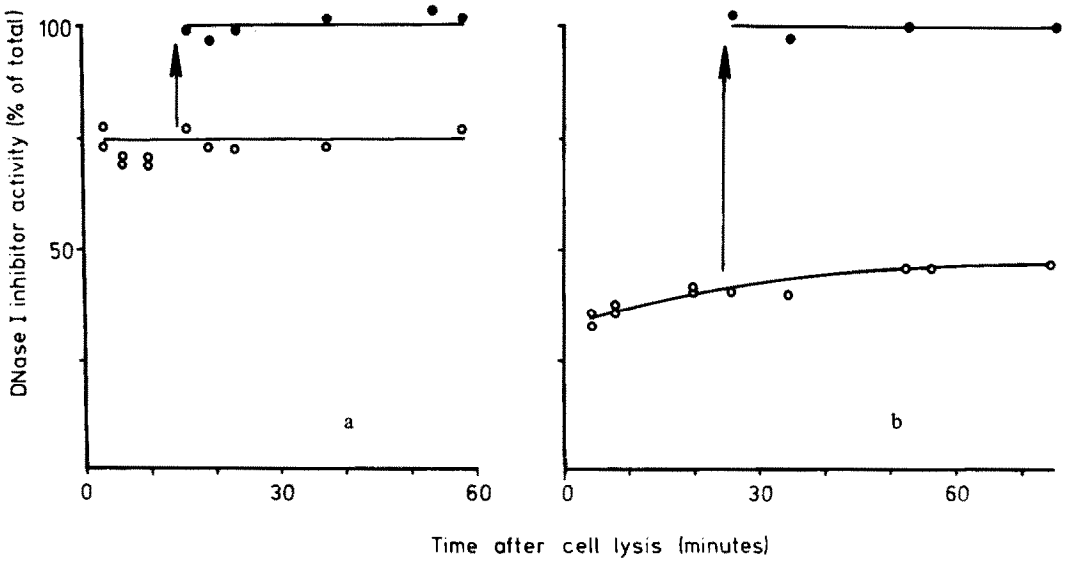

Fig.1. DNase I inhibitor activity in extracts of PCC3 and PCD3 cells. Extracts of (a) PCC3 and (b) PCD3 cells were prepared and assayed for inhibition of DNase I as in section 2. Extracts were kept on ice throughout the experiment. Results are expressed as the activity measured directly (○, representing unpolymerized actin) as a % of the mean of determinations after guanidine hydrochloride treatment of the extract (●, representing the total actin). The 100% values were 33 100 units/ml for PCC3 and 24 800 units/ml for PCD3 extracts, where 1 unit inhibitor activity is defined as that amount giving 1% inhibition of the standard amount of DNase I. Inhibitor at 30 000 units/ml corresponds roughly to actin at 0.3 mg/ml [14].

It would be tempting to correlate the measurements of actin pool in extracts with the results of immunofluorescence studies on the differentiation of EC cells [7], and to draw the conclusion that the diffuse staining of undifferentiated cells represents a pool of unpolymerized actin in the cell. However, it is equally possible that the diffuse staining arises from individual actin filaments which are not resolved in the light microscope and which depolymerize immediately upon cell lysis, giving the high proportion of unpolymerized actin in the extracts. Neither immunofluorescence studies nor the pool analyses presented here can determine the organizational state of actin within the cells, but it is clear that measurement of actin distribution by the DNase inhibition assay provides a quantitative indicator of the reorganization of actin accompanying differentiation.

The basic protein profilin is known to inhibit polymerization of actin in vitro, and has been observed in a complex with unpolymerized actin from a variety of sources [15–18]. In preliminary experiments to characterize the unpolymerized actin pools in extracts of PCC3 and PCD3 cells, lysates were centrifuged at high speed to remove filamentous actin, and the supernatants analysed by chromatography on Sephadex G-100 in lysis buffer without Triton X-100 (not illustrated). Most of the actin, detected both by DNase inhibitor activity and by SDS–polyacrylamide gel electrophoresis, eluted close to the position expected for monomeric actin. Electrophoretic analysis also revealed the presence in extracts of both PCC3 and PCD3 cells of a protein which coeluted with actin from the columns and comigrated with profilin from calf spleen (mol. wt 16 000) on the gels. The unpolymerized state of at least part of the actin in extracts of PCC3 and PCD3 cells thus seems to be maintained by combination with a profilin-like protein. A general role has been suggested for profilin as a regulator of the polymerization state of actin in non-muscle cells [11], so that actin filaments would be formed from a profilin–actin complex in response to specific signals. It should be of interest to investigate what role, if any, is played by profilin in the reorganization of actin during differentiation of EC cells.

Acknowledgements

We thank Francois Jacob, Uno Lindberg, Lars Carlsson and Ingrid Blikstad for discussions, and Borivoj Keil in whose laboratory part of this work was done. We also acknowledge Denise Paulin for providing PCD3 cells and for polyacrylamide gel analyses. F.M. is supported by the Swedish Cancer Research Society and was a recipient of an International Cancer Research Technology Transfer Fellowship from International Union against Cancer, Geneva.

References

[1] Pierce, G. B. and Beals, T. F. (1964) Cancer Res. 24, 1553.
[2] Bernstine, E. G., Hooper, M. L., Grandchamp, S. and Ephrussi, B. (1973) Proc. Natl. Acad. Sci. USA 70, 3899.
[3] Kleinsmith, L. J. and Pierce, G. B. (1964) Cancer Res. 24, 1544.
[4] Artz, K., Dubois, P., Bennett, D., Condamine, H., Babinet, C. and Jacob, F. (1974) Proc. Natl. Acad. Sci. USA 71, 811.
[5] Nicolas, J. F., Dubois, P., Jacob, H., Gaillard, J. and Jacob, F. (1975) Ann. Microbiol. (Inst. Pasteur) 126A, 3.
[6] Jakob, H., Dubois, P., Eisen, H. and Jacob, F. (1978) CR Acad. Sci. Paris 286, 109.
[7] Paulin, D., Nicolas, J. F., Yaniv, M., Jacob, F., Weber, K. and Osborn, M. (1978) Dev. Biol. 66, 213.
[8] Soriano, L. and Paulin, D. (1978) Biochem. Biophys. Res. Commun. 83, 406.
[9] Soriano, L. and Paulin, D. (1980) J. Emb. Exp. Morph. in press.
[10] Goldman, R. D. and Knipe, D. M. (1972) Cold Spring Harbor Symp. Quant. Biol. 57, 263.
[11] Lindberg, U., Carlsson, L., Markey, F. and Nyström, L.-E. (1979) Meth. Achiev. Exp. Pathol. 8, 143.
[12] Markey, F. and Lindberg, U. (1979) Protides of the Biological Fluids (Peeters, H. ed) p. 487, Pergamon, Oxford.
[13] Carlsson, L., Markey, F., Blikstad, I., Persson, T. and Lindberg, U. (1980) Proc. Natl. Acad. Sci. USA in press.
[14] Blikstad, I., Markey, F., Carlsson, L., Persson, T. and Lindberg, U. (1978) Cell 15, 935.
[15] Carlsson, L., Nyström, L.-E., Sundkvist, I., Markey, F. and Lindberg, U. (1977) J. Mol. Biol. 115, 465.
[16] Markey, F., Lindberg, U. and Eriksson, L. (1978) FEBS Lett. 88, 75.
[17] Reichstein, E. and Korn, E. D. (1979) J. Biol. Chem. 254, 6174.
[18] Blikstad, I., Sundkvist, I. and Eriksson, S. (1980) submitted.
[19] Jakob, H., Boon, R., Gaillard, J., Nicolas, J. F. and Jacob, F. (1973) Ann. Microbiol. (Inst. Pasteur) 124B, 269.
[20] Nicolas, J. F., Avner, P., Gaillard, J., Guenet, J. L., Jakob, H. and Jacob, F. (1976) Cancer Res. 36, 4224.