Vol. 105, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 30, 1982 Pages 730-736

LOW-IONIC STRENGTH INDUCES DEGRADATION OF VIMENTIN IN CULTURED HUMAN FIBROBLASTS

I. Virtanen[1], T. Vartio[1,2] and V.-P. Lehto[1]

Departments of Pathology[1] and Virology[2],
University of Helsinki, Haartmanink. 3,
SF-00290 Helsinki 29,Finland

Received February 15, 1982

SUMMARY: Substratum-adherent cytoskeletons, containing vimentin and actin, remain after extraction of cultured human fibroblasts with 0.5 % Triton X-100 in 50 mM Tris-HCl buffer. On the other hand, extraction with Triton X-100 in a 10 mM Tris-HCl buffer, brings about degradation of the vimentin (58 kD) polypeptide with the appearance of an antigenically related 48 kD degradation product, found both in the cell residue and extract. The degradation could be prevented by protease inhibitors but only partially with EDTA. After extraction of cells with 0.5 % Triton X-100/50 mM Tris-HCl a partial degradation of vimentin polypeptide could be obtained upon further extraction in the low ionic strength medium. Detergent extraction in the low ionic medium resulted into a rapid loosening and detachment of most of the nuclei from the growth substratum. The results indicate the presence of a cytoskeleton-associated vimentin-degrading protease in cultured fibroblasts, which may play a role also in the turnover of intermediate filaments.

Five immunologically and biochemically distinct types of intermediate filaments have been found in cytoskeletons of different types of cells in vivo and in vitro (1-4). Cultured fibroblasts contain only vimentin-type of intermediate filaments, consisting of a main 58 kD subunit protein (cf. 1-8). Regulation of the intermediate filament organization is still poorly understood, although recently both phosphorylation (9,10) and a specific proteolytic activity (11-13) have been proposed to be involved. Unlike other cytoskeletal proteins, intermediate filaments seem to lack a significant cytoplasmic pool of soluble subunit protein (cf. 6-8) and they also maintain their integrity during mitosis (cf. 14,15).

0006-291X/82/060730-07$01.00/0

In this report we show that detergent extraction in low ionic milieu is sufficient to induce degradation of vimentin in cultured human fibroblasts. The degradation is apparently protease-mediated as it could be inhibited by inhibitors of proteolysis.

MATERIALS AND METHODS

Human embryonal fibroblasts were cultured in RPMI 1640 medium supplemented with 10 % fetal calf serum (Gibco, Irvine, Scotland) and antibiotics. For light and fluorescence microscopy the cells were cultured on small glass coverslips and fixed in -20°C methanol.

For cytoskeletal extractions (16,17) the cells were carefully washed in NaCl-P buffer (140 mM NaCl, 10 mM sodium phosphate, pH 7.4), scraped off with a rubber policeman and suspended in the extraction medium either at 0°C or at 22°C for 30 min. Triton X-100 was dissolved (0.5 %) in either 10 mM or 50 mM Tris-HCl buffer, pH 7.4, with or without 2 mM $CaCl_2$, phenylmethyl sulfonyl fluoride (PMSF) and 1-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK). After each extraction the cells were pelleted at 12000 xg for 15 min with an Eppendorf centrifuge.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate was performed according to Laemmli (18) using 8 % slab gels. For immunoblotting, the technique of Towbin et al. (19) was used. Briefly, polypeptides were transferred from polyacrylamide gels to nitrocellulose sheets using a commercial apparatus (Bio Rad, Richmond, Ca.). The sheets were then exposed to anti-vimentin antibodies (4), followed by swine anti-rabbit antibodies (Dako, Copenhagen, Denmark) and rabbit peroxidase-anti peroxidase complex (Dako) as described earlier in detail (20). The peroxidase reaction was developed according to Towbin et al. (19).

Indirect immunofluorescence microscopy (IIF) was done with rabbit anti-vimentin antibodies as described earlier (4).

RESULTS

Extraction of the adherent fibroblasts with 0.5 % Triton X-100 in 50 mM Tris-HCl left cytoskeletal preparations which remained anchored to the growth substratum: prominent nuclear residues were visible in phase contrast microscopy (Fig. 1a) and a fine fibrillar organization of vimentin was seen in IIF (Fig. 1b). On the other hand, extraction of the cells with 0.5% Triton in 10 mM Tris-HCl produced cell residues, mostly lacking nuclei, and showing a diffuse granular vimentin specific staining in IIF (Figs 1c,d). When monitored in phase contrast microscopy,

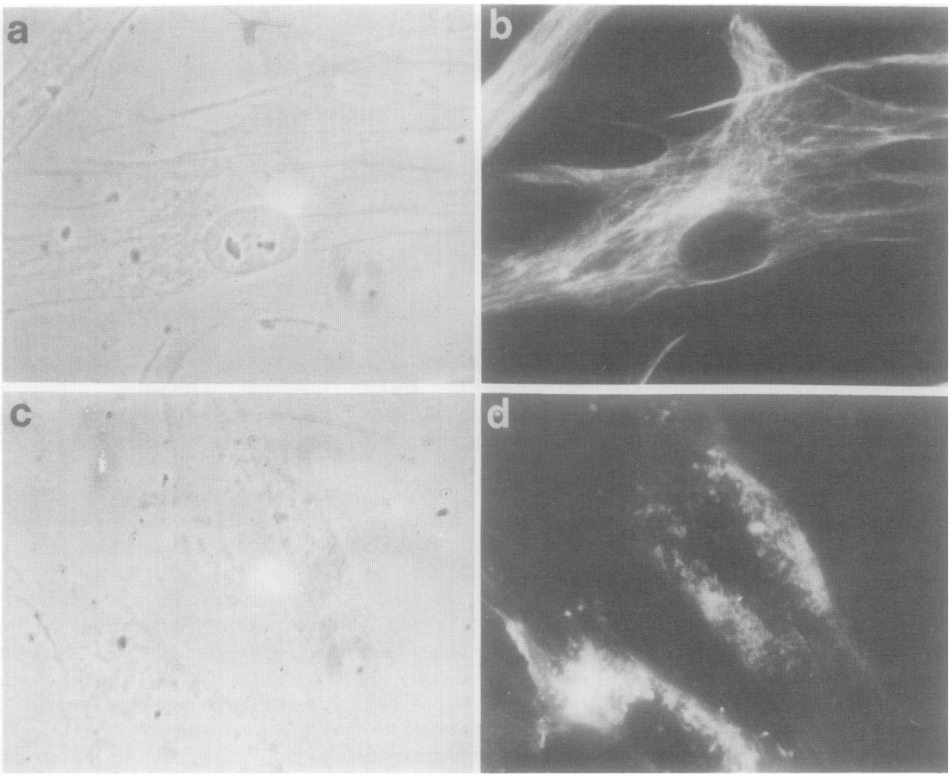
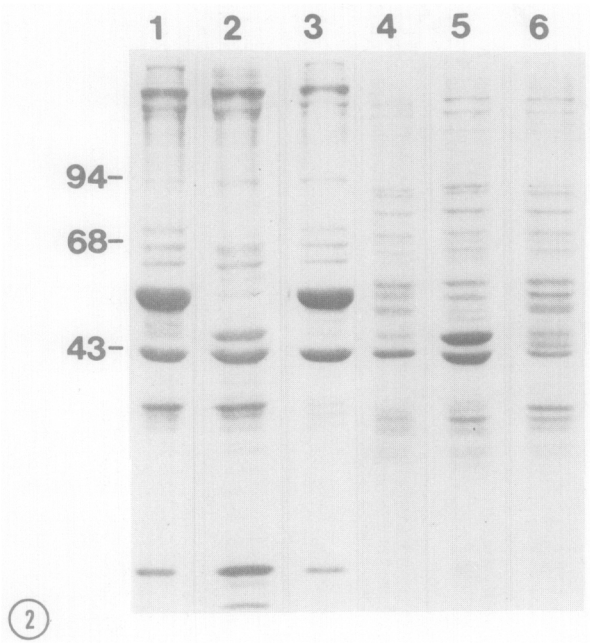


Figure 1. Phase contrast microscopy (a,c) and indirect immunofluorescence microscopy with anti-vimentin antibodies (b,d) of cultured fibroblasts extracted with 0.5 % Triton X-100 in 50 mM Tris-HCl (a,b) or with 0.5 % Triton X-100 in 10 mM Tris-HCl (c,d). Note the fine fibrillar organization of vimentin in b and the diffuse granular distribution in d. x 560.

most of the nuclei were detached during the first minutes of extraction.

In polyacrylamide gel electrophoresis, the 58 kD polypeptide, vimentin (1-4), and actin were revealed as the major cytoskeletal proteins in 0.5 % Triton X-100-50 mM Tris-HCl-extracted cells (Fig. 2, lane 1). The 58 kD polypeptide could not be detected in the extraction medium (Fig. 2, lane 4). On the other hand, extraction of the cells with Triton X-100 in 10 mM Tris-HCl produced cell residues containing actin and a 48 kD polypeptide as the major polypeptides (Fig. 2, lane 3). A similar 48 kD polypeptide was seen as a prominent band also in the cell extract while both the cell residues and extract



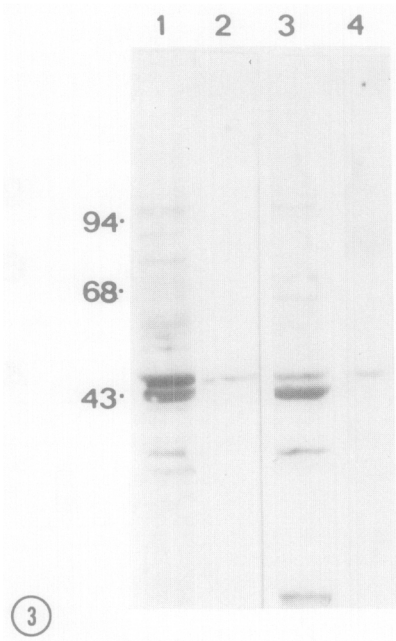


Figure 2. Polyacrylamide gel electrophoresis of cultured human fibroblasts extracted with 0.5 % Triton X-100 in 50 mM Tris-HCl (lanes 1,4), with 0.5 % Triton X-100 in 10 mM Tris-HCl (lanes 2,5) and with 0.5 % Triton X-100 in 10 mM Tris-HCl, supplemented with 2 mM TPCK (lanes 3,6). Lanes 1-3 represent the cell residues and lanes 4-6 the cell extracts. Coomassie blue staining. Note the prominent 58 kD vimentin polypeptide in cell residues in lanes 1 and 3 and the prominent 48 kD polypeptide both in cell residue and cell extract in lanes 2 and 5.

Figure 3. Immunoblotting of the cell residue (lane 2) and the extract (lane 4) with anti-vimentin antibodies after extraction of cultured fibroblasts with 0.5 % Triton X-100 in 10 mM Tris-HCl. Lanes 1 and 3 show amido-black stained blots of the cell residue (1) and the cell extract (3), respectively. Note the distinct peroxidase reaction with the 48 kD polypeptide.

lacked the 58 kD polypeptide (Fig. 2, lanes 2,5). In immunoblotting the 48 kD polypeptide from both the cell residues and extract showed a reaction with anti-vimentin antibodies (Fig. 3, lanes 1 to 4), indicating a precursor-degradation product relationship between the 58 kD and the 48 kD polypeptides.

The degradation of the 58 kd polypeptide could be totally inhibited by inclusion of TPCK (Fig. 2, lanes 3,6), but not PMSF, in the culture medium but only partially with EDTA (Fig. 4, lane 1). Interestingly, addition of 2 mM $CaCl_2$ in the extraction medium had no apparent effect on the integrity of

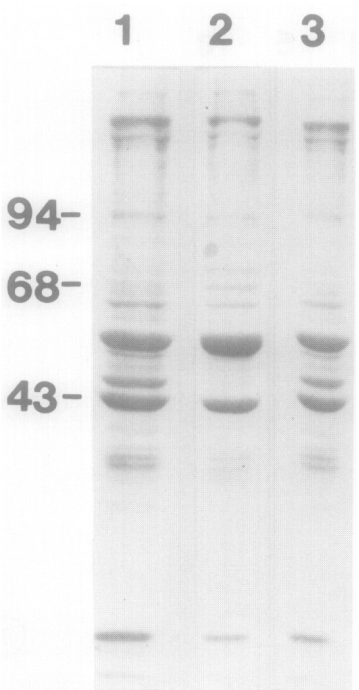


Figure 4. Polypeptide analysis of the cell residues after extraction of human fibroblasts with 0.5 % Triton X-100 in 10 mM Tris-HCl supplemented with 2 mM EDTA (1), after extraction with 0.5 % Triton X-100 in 50 mM Tris-HCl supplemented with 2 mM $CaCl_2$ (2) and after extraction with first 0.5 % Triton X-100 in 50 mM Tris-HCl, followed by 10 mM Tris-HCl (lane 3).

vimentin in 50 mM Tris-HCl medium (Fig. 4, lane 2). When human fibroblasts were first extracted with 0.5 % Triton X-100 in 50 mM Tris-HCl and then with the low ionic Triton-buffer, a partial degradation of the 58kd polypeptide could be detected (Fig. 4, lane 3).

DISCUSSION

The intermediate filament-forming 58 kD polypeptide of fibroblasts, vimentin (1), found also in cytoskeletons of other types of cultured cells (1-8), shares some unusual properties with other intermediate filament proteins (cf. for review 3,8,21). It is highly insoluble within a wide pH and ionic strength range, and no soluble subunit protein can be found in nonionic detergent extracts of cultured fibroblasts (cf. 6-8).

Recently, a Ca^{++}-activated cytoplasmic protease has been suggested to play a role in regulation of the organization of vimentin filaments in some cultured cells (11,13). A related protease activity has been found also in glial and neuronal cells (22,23). The results of the present study show that a complete degradation of the 58 kD vimentin polypeptide can be achieved by extracting cultured fibroblasts with a nonionic detergent in a low ionic medium. Degradation did not take place in a higher ionic strenght than 10 mM even in the presence of Ca^{++} but it is apparently protease-mediated as indicated by the inhibiting effect of TPCK. These results suggest that other factors than the presence of Ca^{++} may play the major role in the activation of the vimentin-degrading protease in cells (but see 13,15). Interestingly, degradation of vimentin could be achieved also upon further extraction of Triton-cytoskeletons in the low ionic medium, suggesting that the protease activity is closely associated with intermediate filaments rather than located in the cytoplasm as suggested recently (cf. 13,15).

Earlier studies have suggested that intermediate filaments in fibroblasts consist of protofilaments (eg. 8,24). Moreover, both Steinert et al. (25) and Zackroff and Goldman (26) have shown that the protofilament organization of reconstituted vimentin filaments disassembles slowly in low ionic milieu. Such a change in the intermediate filament organization may also result in the activation of the vimentin degradation as shown in this study.

Acknowledgements

The skillful technical assistance of Ms. Pipsa Kaipainen and Ms. Raili Taavela is kindly acknowledged. This study was supported by grants from Finnish Medical Research Council and the Association of Finnish Life Insurance Companies.

REFERENCES

1. Franke, W., Schmid, E., Osborn, M., and Weber, K. (1978). Proc. Natl. Acad. Sci. USA 75, 5034-5038.
2. Bennett, G.S., Fellini, S.A., Croop, J.M., Otto, J.J., Bryan, J., and Holtzer, H. (1978). Proc. Natl. Acad. Sci. USA 75, 4364-4368.
3. Lazarides, E. (1980). Nature 283, 249-256.
4. Virtanen, I., Lehto, V.-P., Lehtonen, E., Vartio, T., Stenman, S., Kurki, P., Wager, O., Small, J.V., Dahl, D., and Badley, R.A. (1981) J. Cell Sci. 50, 45-63.
5. Franke, W.W., Schmid, E., Winter, S., Osborn, M., and Weber, K. (1979). Exp. Cell Res. 123, 25-46.
6. Albertini, D.F., and Kravit, N.G. (1981). J. Biol. Chem. 245, 2484-2492.
7. Cabral, R., Willingham, M.C., and Gottesman, M.M. (1980). J. Histo. Cytochem. 28, 653-662.
8. Renner, W., Franke, W.W., Schmid, E., Geisler, N., Weber, K., Mandelkov, E. (1981). J. Mol. Biol. 149, 285-306.
9. Browning, E.T., and Sanders, M.M. (1981). J. Cell Biol. 90, 803-808.
10. Robinson, S.I., Nelkin, B., Kaufmann, S., and Vogelstein, B. (1981). Exp. Cell Res. 133, 445-449.
11. Nelson, W.J., and Traub, P. (1981). Eur. J. Biochem. 116, 51-57.
12. Ocks, D.C., McConkey, E.H., and Guard, N.L. (1981). Exp. Cell Res. 135, 355-362.
13. Traub, P., and Nelson, W.J. (1981). Eur. J. Cell Biol. 26, 61-67.
14. Aubin, J.E., Osborn, M., Franke, W.W., and Weber, K. (1980). Exp. Cell Res. 129, 149-165.
15. Blose, S.H. (1979). Proc. Natl. Acad. Sci. USA 76, 3372-3376.
16. Lehto, V.-P., Virtanen, I., and Kurki, P. (1978). Nature 272, 175-177.
17. Lehto, V.-P., Vartio, T., and Virtanen, I. (1980). Biochem. Biophys. Res. Commun. 95, 909-916.
18. Laemmli, U.K. (1970). Nature 227, 680-683.
19. Towbin, H., Staehelin, T., and Gordon, J. (1979). Proc. Natl. Acad. Sci. USA 76, 4350-4354.
20. Virtanen, I., von Koskull, H., Lehto, V.-P., Vartio, T., and Aula, P. (1981). J. Clin. Invest. 68, 1348-1355.
21. Anderson, B.H. (1981). J. Muscle Res. Cell Motil. 2, 141-166.
22. Schlaepfer, W.W., and Micko, S. (1979). J. Neurochem. 32, 211-219.
23. Schlaepfer, W.W., and Zimmerman, O.-J. P. (1981). Neurochem. Res. 6, 243-255.
24. Franke, W.W., Zerban, H., Grund, C., and Schmid, E. (1981). Biol. Cell 41, 173-178.
25. Steinert, P.M., Idler, W.W., Cabral, F., Gottesman, M.M., and Goldman, R.D. (1981). Proc. Natl. Acad. Sci. USA 78, 3692-3696.
26. Zackroff, R.V., and Goldman, R.D. (1969). Proc. Natl. Acad. Sci. USA 76, 6226-6230.