Vol. 84, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 14, 1978 Pages 232-237

ε-(γ-GLUTAMYL)LYSINE ISOPEPTIDE BONDS IN NORMAL AND VIRUS TRANSFORMED HUMAN FIBROBLASTS

Paul J. Birckbichler, Henry A. Carter, Gerald R. Orr,

E. Conway, and M. K. Patterson, Jr.

Biomedical Division, The Samuel Roberts Noble Foundation, Inc., Ardmore, Oklahoma 73401

Received July 12,1978

SUMMARY

Normal human lung cells (WI-38) possessed 20-40 times more ε-(γ-glutamyl)lysine isopeptide bonds than Simian virus transformed counterparts, WI-38 VA13A and WI-38 VA13-2RA. Normal cells arrested in an essentially nonmitotic state had more isopeptide bonds than proliferating cells. Isopeptide content paralleled the transglutaminase activity of these cells. The results suggest that isopeptide crosslinks contribute to a cellular architecture conducive to a nonproliferating state.

INTRODUCTION

Isopeptides of the type ε-(γ-glutamyl)lysine exist in a variety of biological materials (1-3) including membrane proteins of eukaryotic cells (4). Formation of such isopeptides is postulated to occur via enzymic catalysis by a group of enzymes called transglutaminases (5, 6). However, a direct correlation between isopeptide product and transglutaminase activity is lacking in most systems (1).

Transglutaminase activity is reduced in several cell types following viral and chemical transformation of cells in tissue culture (7), in chemically-induced rat hepatomas (8), and in mitogen-induced lymphoblasts and lymphoblastic cell lines (9). Because of this difference in transglutaminase activity and its apparent implications in the transformed state, it was of interest to determine if the isopeptide content was correspondingly different

0006-291X/78/0841-0232$01.00/0

in normal and transformed cells. The results reported here demonstrate reduced levels of isopeptide following viral transformation of human lung cells. Differences in isopeptide content were also detected between proliferating and nonproliferating normal cells. This communication describes one of the few instances where an apparent correlation is established between transglutaminase activity and enzyme reaction product, ε-(γ-glutamyl)lysine.

MATERIALS AND METHODS

Human diploid embryonic lung cells, WI-38, were purchased from the American Type Culture Collection, Rockville, MD, and passages 25 to 37 were used. SV40-transformed WI-38 VA13A and WI-38 VA13-2RA were supplied by Dr. V. J. Cristofalo of The Wistar Institute, Philadelphia, PA and Dr. A. J. Girardi of the East Tennessee Cancer Research Center, Knoxville, TN.

Cell cultures were maintained in T-75 flasks (Corning Glass Works, Corning, NY) by medium changes every 48 hr using modified McCoy's Medium 5a (7) supplemented with 10% fetal bovine serum (Reheis Chemical Corporation, Phoenix, AZ). Essentially nonmitotic populations of cells were obtained by lowering the serum concentration in the medium to 0.5% seven days after subculture (10). Under these conditions the mitotic indices at day 3, 7, and 14 following subculture were 3.5%, 0.2%, and 0%, respectively (11). Cultures were negative for mycoplasma by monthly tests (12).

ε-(γ-Glutamyl)lysine isopeptide was determined on total cell protein after exhaustive enzyme digestion by pronase under sterile conditions. After removal of medium, replicate flasks were rinsed three times with calcium-free Earle's solution. One flask was treated with 0.1 M citric acid and aliquots were taken for cell enumeration (13), protein quantitation (14), and hydrolysis for 18 hr in boiling 6 N hydrochloric acid. The acid hydrolyzate was subsequently analyzed for amino acid composition by ion exchange chromatography (15) to calculate total recovery. The remaining flasks were incubated at 37° C with 5 ml of 10 mM Tris-HCl, pH 7.5, containing 1 mg pronase (Calbiochem, La Jolla, CA). An additional 1 mg pronase was added at 48 and 96 hr. After 168 hr of incubation, trichloroacetic acid was added to a final concentration of 10% and the precipitate that formed was removed by centrifugation. The liquid was processed for isopeptide by high pressure liquid chromatography as described previously (15) with the following modifications. The radioactive fractions from the HP-B80 resin column were collected, lyophilized, dissolved in 0.1 N hydrochloric acid, and rechromatographed prior to analysis on a Glenco amino acid analyzer. In some instances the rechromatographed sample was dissolved in 0.1 N hydrochloric acid instead of pH 2.2 sodium citrate buffer. This was done to facilitate further analysis of portions of the sample without the need of desalting again. The hydrochloric acid did not significantly alter the retention time or resolution of the isopeptide on the Glenco column.

TABLE 1

ISOPEPTIDE CONTENT IN NORMAL AND TRANSFORMED HUMAN LUNG CELLS

Cell	Days After Subculture	Transglutaminase Activity[a] (units/mg protein)	e-(γ-Glutamyl)lysine	
			(pmole/10^6 cells)	(pmole/mg protein)
Normal				
WI-38	7	8.4 ± 0.9 [b] (5)[c]	699 ± 81 (9)	2430 ± 281
Transformed				
WI-38 VA13A	7- 9	0.12 ± 0.02 (7)	19 ± 4 (10)	71 ± 16
WI-38 VA13-2RA	8-11	0, 0.08	34, 37	81, 120

[a] Data taken from reference 7.

[b] Mean ± SE.

[c] Number in parentheses = number of preparations assayed.

RESULTS AND DISCUSSION

Table 1 illustrates that normal human lung cells possessed 20-40 times more e-(γ-glutamyl)lysine isopeptide bonds than transformed counterparts, WI-38 VA13A and WI-38 VA13-2RA. The differences were evident whether expressed on a per cell or per mg of protein basis. These differences in isopeptide content are consistent with differences we previously reported in transglutaminase activity (7) and in immunofluorescent staining with antibody to purified transglutaminase (16).

The level of isopeptide crosslinks, like the enzyme activity in the normal cell (7), appeared to reflect the proliferative state (Table 2). Fewest crosslinks were seen in rapidly proliferating preconfluent cultures (day 3). As cultures reached confluency (day 7) and more of the cells entered the nonproliferative state, the level of isopeptide increased 2- to 3-fold. The level of isopeptide was also significantly higher after the cells were in the arrested state for 7 days (day 14). This increase was blocked

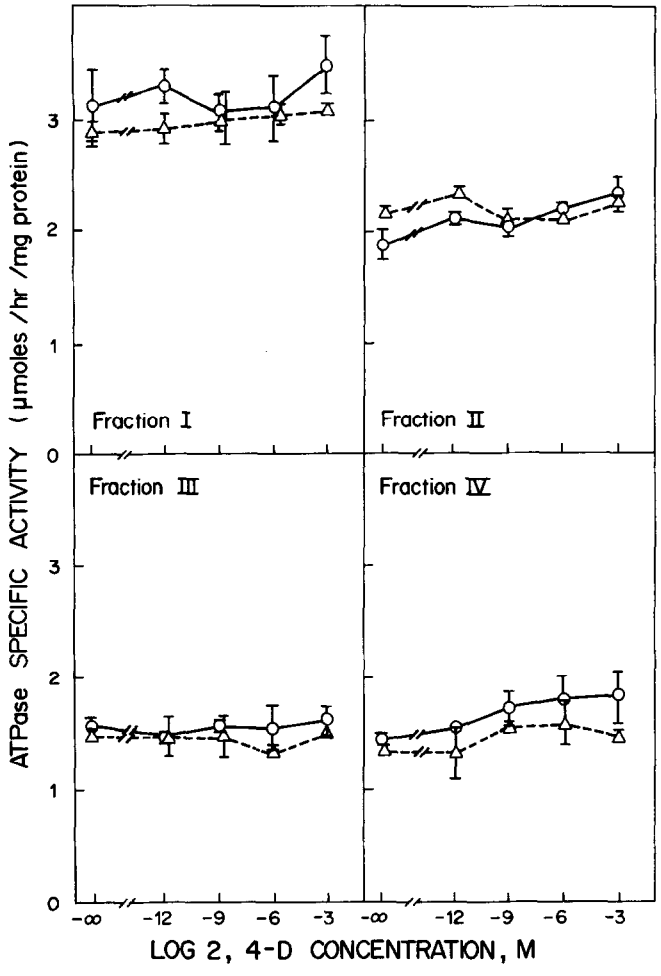

Figure 2. Modulation of ATPase activity by 2,4-D at pH 6.0. Assays contained 50 mM Tris and 135 μM nupercaine. Circles: without potassium chloride; triangles: with 50 mM potassium chloride.

Endogenous phosphate release was also observed to be stimulated up to 30% by 2,4-D over a broad range of hormone concentrations (Fig. 4). The absolute levels of the endogenous activity were variable among different experiments.

In contrast to ATPase, phosphatidate phosphatase was inhibited by 2,4-D as other phosphatase activities were stimulated (Fig. 5). The inhibition of phosphatidate phosphatase ranged from 15-25% in membranes isolated and incubated in the presence of nupercaine. Acid phosphatase activity in the same membrane preparation and under the same incubation conditions was not influ-

tide crosslinks of the cellular architecture and a nonproliferating state. Since membrane proteins contain e-(γ-glutamyl)lysine bonds (4), the crosslinking of membrane proteins, integral or peripheral, may be a method of membrane stabilization. Activation of erythrocyte "intrinsic" transglutaminase has been shown to produce polymeric membrane proteins containing isopeptide bonds (18-20). Attempts to localize and identify the specific protein(s) involved in isopeptide bond formation in the human lung cell are currently in progress.

REFERENCES

1. Folk, J. E., and Finlayson, J. S. (1977) in "Advances in Protein Chemistry" (C. B. Anfinsen, J. T. Edsall, and R. M. Richards, eds.), Vol. 31, pp. 1-133, Academic Press, Inc., New York.
2. Abernethy, J. L., Hill, R. L., and Goldsmith, L. A. (1977) J. Biol. Chem., 252, 1837-1839.
3. Rice, R. H., and Green, H. (1977) Cell, 11, 417-422.
4. Birckbichler, P. J., Dowben, R. M., Matacić, S., and Loewy, A. G. (1973) Biochim. Biophys. Acta, 291, 149-155.
5. Folk, J. E., and Chung, S. I. (1973) in "Advances in Enzymology" (A. Meister, ed.), Vol. 38, pp. 109-191, Academic Press, Inc., New York.
6. Lorand, L., and Stenberg, P. (1976) in "Handbook of Biochemistry and Molecular Biology. Proteins" (G. D. Fasman, ed.), Vol. II, pp. 669-685, CRC Press, Cleveland, Ohio.
7. Birckbichler, P. J., Orr, G. R., Conway, E., and Patterson, M. K., Jr. (1977) Cancer Res., 37, 1340-1344.
8. Birckbichler, P. J., Orr, G. R., and Patterson, M. K., Jr. (1976) Cancer Res., 36, 2911-2914.
9. Novogrodsky, A., Quittner, S., Rubin, A. L., and Stenzel, K. H. (1978) Proc. Natl. Acad. Sci. (U.S.A.), 75, 1157-1161.
10. Dell'Orco, R. T., Mertens, J. G., and Kruse, P. F., Jr. (1973) in "Tissue Culture: Methods and Applications" (P. F. Kruse, Jr., and M. K. Patterson, Jr., eds.), p. 231, Academic Press, New York.
11. Dell'Orco, R. T., Mertens, J. G., and Kruse, P. F., Jr. (1973) Exptl. Cell Res., 77, 356-360.
12. Barile, M. F. (1973) in "Tissue Culture: Methods and Applications" (P. F. Kruse, Jr., and M. K. Patterson, Jr., eds.), pp. 729-735, Academic Press, Inc., New York.
13. Absher, M. (1973) in "Tissue Culture: Methods and Applications" (P. F. Kruse, Jr., and M. K. Patterson, Jr., eds.), pp. 395-397, Academic Press, Inc., New York.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem., 193, 265-275.

15. Birckbichler, P. J., Orr, G. R., Carter, H. A., and Patterson, M. K., Jr. (1977) Biochem. Biophys. Res. Commun., 78, 1-7.
16. Birckbichler, P. J., and Patterson, M. K., Jr. (1978) Ann. N. Y. Acad. Sci., in press.
17. Birckbichler, P. J., Orr, G. R., Carter, H. A., Conway, E., Maxwell, M. D., and Patterson, M. K., Jr. (1978) Federation Proc., 37, 1598.
18. Lorand, L., Weissmann, L. B., Epel, D. L., and Bruner-Lorand, J. (1976) Proc. Natl. Acad. Sci. (U.S.A.), 73, 4479-4481.
19. Anderson, D. R., Davis, J. L., and Carraway, K. L. (1977) J. Biol. Chem., 252, 6617-6623.
20. Siefring, G. E., and Lorand, L. (1978) "Progress in Clinical and Biological Research" (W. C. Kruckenberg, J. W. Eaton, and G. J. Brewer, eds.), Vol. 20, pp. 25-32, Alan R. Liss, Inc., New York.