Vol. 135, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 26, 1986 Pages 304-308

BIOCHEMICAL AND IMMUNOLOGIC STUDIES ON THE NATIVE AND DEGLYCOSYLATED FORMS OF γ -GLUTAMYLTRANSPEPTIDASE OF RAT KIDNEY

Julio Coloma and Henry C. Pitot

McArdle Laboratory for Cancer Research
The Medical School
University of Wisconsin
Madison, Wisconsin 53706

Received January 9, 1986

We have deglycosylated the enzyme gamma-glutamyl transpeptidase by treatment of the protein with anhydrous hydrofluoric acid at 0°C. After deglycosylation, the heavy and light subunits showed a molecular weight of 43 and 23 Kd respectively. Whereas the antiserum against the native enzyme recognized both proteins, the antiserum against the deglycosylated enzyme failed to recognize the native enzyme, indicating that some of the determinants of the native enzyme are masked by the carbohydrate moiety.

Gamma-glutamyltranspeptidase (GGT, EC 2.3.2.2) is a glycosylated, membrane-associated enzyme ubiquitously found in most mammalian tissues (1). The native enzyme isolated from rat kidney is more than 19% carbohydrate in its molecular form (2,3). The structure of the carbohydrate moieties of rat kidney gamma-glutamyltranspeptidase are extremely heterogeneous. Furthermore, while the polypeptide portions of the molecule isolated from several different tissues including liver, intestine (4), and hepatoma (5) are similar if not identical, the carbohydrate moieties of the enzymes from these various tissue sources are significantly different in structure (4-6). As yet, however, there have been no published studies on the characteristics of the deglycosylated form of the enzyme. This paper describes a method for deglycosylation of the enzyme and some of its immunologic characteristics.

MATERIALS AND METHODS

GGT was purified from rat kidney after solubilization with Triton X-100 according to the procedure of Hughey and Curthoys (7) with minor modifications. The purity of the different batches was determined by SDS-PAGE and HPLC.

0006-291X/86 $1.50

Deglycosylation of GGT was carried out by treatment of the protein with anhydrid hydrofluoric acid (HF) in presence of anisol as scavenger (8,9) using an apparatus constructed in Teflon Kel-F (9). The reaction was allowed to proceed at 4°C for 60 minutes. After deglycosylation and removal of all unreacted HF in vacuo the protein was dissolved in glacial acetic acid and stored at -20°C.

Antibodies against the native and deglycosylated enzyme were raised in rabbits by standard procedures. Due to the insolubility of the deglycosylated GGT, the protein was precipitated from the acetic acid solution by neutralization with NaOH and resuspended in 0.15 M NaCl prior to injection. Antibodies against the native enzyme were affinity purified on a GGT-Sepharose 4B column (10). Antibodies against the deglycosylated enzyme were partially purified by salting out with $(NH_4)SO_4$ (11).

Immunoblot procedure. After electrophoresis in SDS-polyacrylamide gel, proteins were transferred to nitrocellulose filters (12) and identified using their specific antibodies followed by localization with antiglobulin-immunoperoxidase complexes (13).

Chemicals. Goat anti-rabbit IgG, horseradish peroxidase-rabbit anti horseradish peroxidase complex and 3-3'diaminobenzimide were purchased from Sigma (St. Louis, MO). Nitrocellulose filters (BA84, 0.45μ) were from Schleicher and Schuell. Other reagents were of the highest quality available.

RESULTS

Attempts to deglycosylate GGT enzymatically failed, due in part to the fact that highly purified endo- and exoglycosidases must be used in the appropriate sequence and to the presence of bisect N-acetylglucosamine residues which make GGT partially resistent to some of those enzymes (14). Chemical deglycosylation by periodic acid oxidation is often incomplete, and has the disadvantage that some aminoacid residues are oxidized as well (15). Treatment of proteins with anhydrous HF offers the possibility of cleavage of or most of the linkages of neutral and acidic sugars, without affecting the peptide backbone (8).

When native GGT was subjected to SDS-PAGE, it showed two bands, with apparent molecular weights of 50 kd (heavy subunit) and 30 kd (light subunit) (Figure 1). After treatment with HF the protein yielded two bands with molecular weights of 43 and 23 kd, indicating a molecular weight for the protein backbone of 66 kd. This result agreed with the previous findings of Nash and Tate (2) and Hanoune and coworkers (3) who found a 63 kd peptide by immunoprecipitation from an in vitro translation mixture of kidney poly(A)RNA. This polypeptide was identified as the protein precursor of the enzyme. The difference in the molecular weight may be due to the presence of some residual carbohydrate (8).

The ratio of carbohydrate to protein was the same in both subunits of the native enzymes. Carbohydrate accounted for approximately 22.3% of the native GGT in these studies.

The native and deglycosylated proteins were run in an SDS-polyacrylamide gel and, after transfer to nitrocellulose filters, were probed with antisera raised against both proteins, as described in Materials and Methods. When the antiserum against the native enzyme was used, the two subunits of both proteins showed immunoreactivity (Figure 2A). In general the heavy subunit showed a stronger reaction than the light subunit (result not shown). Higher concentrations of antisera were necessary to make the light subunit of both proteins visible. On the other hand, the antiserum against the deglycosylated enzyme only recognized the deglycosylated protein (Figure 2Bc), showing no reactivity

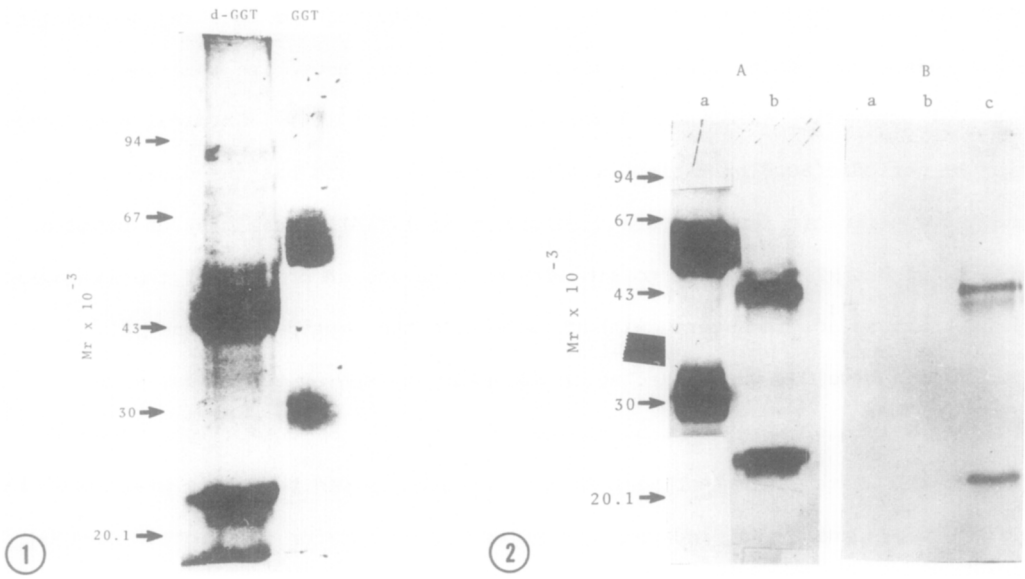

Figure 1. SDS-polyacrylamide gel electrophoresis of native and deglycosylated rat kidney GGT. Proteins were resolved in a 10% polyacrylamide gel and stained with Coomassie blue. Molecular weight markers are indicated by arrows. GGT: gamma-glutamyltranspeptidase. d-GGT: deglycosylated GGT.

Figure 2. Immunoblots of native and deglycosylated GGT. Proteins were run in a 10% SDS-polyacrylamide gel, transferred to nitrocellulose filters and detected using different antisera. A. native GGT antiserum. a: GGT. b: d-GGT. B: d-GGT antiserum. a: kidney homogenate. b: native GGT. c: d-GGT.

with the native enzyme or any other protein in a kidney homogenate (Figure 2Ba,b).

DISCUSSION

The presence of a carbohydrate moiety in glycoproteins is often a source of difficulty when studying a number of their properties. Glycoproteins behave anomolously when subjected to SDS-PAGE, yielding higher molecular weights than expected (16) and broad bands that seem to reflect the complexity of the sugar chains (15). The carbohydrates are a major problem when a specific antibody against the peptide part is needed due to the high antigenicity of the carbohydrate moiety.

In general, the carbohydrate moiety on glycoproteins tends to have several functions. One of these involves the stabilization of the protein, preventing or decreasing metabolic turnover or intracellular proteolysis (18). Specifically, Olden and his associates (19) have suggested that the carbohydrate moiety of surface glycoproteins is not required for their synthesis, secretion, or biological function but instead helps to protect the protein against proteolytic degradation. Such is likely the case for gamma-glutamyltranspeptidase which occurs predominantly as an ectoenzyme on the outer surface of plasma membranes of cells. In the case of gamma-glutamyltranspeptidase, the carbohydrate moiety is not involved in the association of the two subunits by means of covalent linkages between sugar residues since the native enzyme may be dissociated into its subunits by reduction of disulfide bridges (Figure 1). Although antibodies to the protein as well as the carbohydrate moieties of the enzyme can be induced in rabbits, it is clear that some determinants of the protein moiety are "masked" by the sugar residues preventing their availability to the antibody-forming system (Figure 2). Thus, the protective nature of the carbohydrate residues on gamma-glutamyltranspeptidase not only appear to prevent the action of proteolytic factors in degrading the enzyme, but also screen or mask antigenic determinants from the antibody-forming system of the rodent.

ACKNOWLEDGMENTS

Authors are grateful to Dr. D. H. Rich and Dr. N. Agawal of the School of Pharmacy of the University of Wisconsin for allowing us the use of the deglycosylatio apparatus. This work was supported by grants from the National Cancer Institute (CA-07175 and CA-22484). TC's present address is Department of Biochemistry, School of Pharmacy, University of Valencia, Spain.

REFERENCES

1. Vanderlaan, M., and Phares, W. (1981) Histochem. J. 13, 865-877.
2. Nash, B., and Tate, S. S. (1984) J. Biol. Chem. 259, 678-685.
3. Finidori, J., Laperche, Y., Haguenauer-Tsapis, R., Barouki, R., Guellaen, G., and Hanoune, J. (1984) J. Biol. Chem. 259, 4687-4690.
4. Shaw, L. M., Petersen-Archer, L., London, J. W., Marsh, E. (1980) Clin. Chem. 26, 1523-1527.
5. Tsuchida, S., Hoshino, K., Sato, T., Ito, N., and Sato, K. (1979) Cancer Res. 39, 4200-4205.
6. Yamashita, K., Hitoi, A., Taniguchi, N., Yokosawa, N., Tsukada, Y., and Kobata, A. (1983) Cancer Res. 43, 5059-5063.
7. Hughey, R. P., and Curthoys, N. P. (1976) J. Biol. Chem. 251, 7863-7870.
8. Mort, A. J., and Lamport, D. T. A. (1977) Anal. Biochem. 82, 289-309.
9. Manjunath, P., and Sairam, M. R. (1985) Methods in Enzymology 109, pp. 725-735. Academic Press, New York.
10. Cuatrecasa, P., and Anfinsen, C. B. (1971) Methods in Enzymology 22, pp. 345-378. Academic Press, New York.
11. Kendall, F. E. (1937) J. Clin. Invest. 16, 921-929.
12. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA, 76, 4350-4354.
13. Tsang, V. C. W., Peralta, J. M., and Simmons, A. R. (1983) Methods in Enzymology 92, pp. 377-391. Academic Press, New York.
14. Yamashita, K., Hitoi, A., Matsuda, Y., Tsuji, A., Katunuma, N., and Kobata, A. (1983) J. Biol. Chem. 258, 1098-1107.
15. Clamp, J. R., and Hough, L. (1965) Biochem. J. 94, 17-29.
16. Segrest, J. P., Jackson, R. L., Andrews, E. P., and Marchesi, V. T. (1971) Biochem. Biophys. Res. Comm. 44, 390-395.
17. Olden, K., Parent, J. B., and White, S. L. (1982) Biochim. Biophys. Acta 650, 209-232.
18. Olden, K., Bernard, B. A., White, S. L., and Parent, J. B. (1982) J. Cell. Biochem. 18, 313-335.