Volume 216, number 1, 151-154 FEB 04713 May 1987

Differences between individuals in high-M_r glycoproteins from human mammary epithelia

Stuart Patton and Gail E. Huston

Department of Neurosciences (M-008), University of California at San Diego, La Jolla, CA 92093, USA

Received 27 January 1987; revised version received 11 March 1987

SDS-polyacrylamide gel electrophoretic analysis of high molecular mass glycoproteins from human milk fat globules revealed that the pattern of bands is unique for each milk donor. The number, position, width and intensity of bands varied between donors. A difference in the pattern of sisters could be discerned. There is evidence that these mucin-like glycoproteins, which originate from the plasma membrane of the mammary epithelial cell, occur in other cells and tissues. They may function in the immunorecognition system because of their individual-specific character and cell surface location.

Membrane glycoprotein; Immune system; (Mammary epithelial cell, Human milk)

1. INTRODUCTION

Many important events that occur at cell surfaces depend on exteriorized structures of the plasma membrane. The MFG affords a unique opportunity to study such structures because it is enveloped by plasma membrane of the lactating epithelial cell at secretion [1,2]. Recently, Shimizu and co-workers [3,4] have characterized mucin-like high-M_r (HM_r) glycoproteins from the membrane of the human MFG. These proteins appear to have trans-membrane configurations with *O*-linked oligosaccharides projecting from the exocellular surface. Initially, a glycoprotein (PAS-0) containing approx. 50% carbohydrate which binds *Ricinus communis*, wheat germ and peanut agglutinins was reported [3]. This protein was seen to just enter a 5% SDS-polyacrylamide gel. Subsequently, another, even higher-M_r protein (Component A), containing 65–80% carbohydrate was found in this membrane, and PAS-0 was reported to be comprised of two closely related proteins (Components B and C) [4]. While Component A was observed to bind peanut and wheat germ lectins in common with B and C, it was differentiated from the latter two by its binding of several monoclonal antibodies and soybean agglutinin. Although the M_r values of these proteins have not been determined, they are referred to as HM_r because of their restricted mobilities in SDS gels, even those of very low acrylamide contents (2–5%). These mobilities are decidedly less than that of myosin (M_r 200 000) under such conditions.

We present here evidence of differences between individuals in the HM_r glycoproteins of human MFGs.

Correspondence address: S. Patton, Department of Neurosciences (M-008), University of California, San Diego, La Jolla, CA 92093, USA

Abbreviations: PAGE, polyacrylamide gel electrophoresis; MFG, milk fat globule; PAS, periodic acid-Schiff reagents

2. MATERIALS AND METHODS

Fat globules were isolated by centrifuging them out of freshly collected human milk samples through an overlayer of water [5]. Final concentrations of purified globules were adjusted to 10–20% based on total lipid weight determined by the Roese-Gottlieb method [6]. After removal of lipids, relative protein content of the samples was

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

estimated by the procedure of Lowry et al. [7] using bovine serum albumin as standard. In some instances milk samples and water used in globule isolations were treated with the protease inhibitors, ϵ-aminocaproic acid and phenylmethylsulfonyl fluoride, both at concentrations of 100 μM. Globule samples were preserved with 0.02% sodium azide and were stored at $-20°C$.

SDS-PAGE of globule preparations was conducted as described by Laemmli [8]. The samples were conventionally denatured with SDS, mercaptoethanol and heat [8]. They were then centrifuged at 1500 × g for 20 min and only the fat-depleted lower phases were electrophoresed. Slab gels 105 mm long, 135 mm wide and 1.5 mm thick were used. The top 20 mm was a 3% acrylamide stacking gel, the remainder (85 mm) being a 4% running gel. A current of 10 mA, falling to 6 mA, was applied for 18 h. Gels were stained for glycoproteins with PAS or for proteins in general with Coomassie blue according to Fairbanks et al. [9]. Fragility of the gels required that they be moved from one container to another by inversion onto and from a thin, pliable plastic film, that they not be shaken, and that staining and washing solutions be removed by siphoning.

3. RESULTS

SDS-PAGE patterns of the HM_r glycoproteins from MFGs of 8 donors are shown in fig.1. Position, number, width and intensity of bands varied from one sample to another. In some cases there were two bands in the stacking gel (lanes 2,3,5 and 6), in others there were two in the running gel (lanes 1,2,4,7 and 8) and one individual had two in both gels (lane 2). Lanes 7 and 8 enable a comparison of patterns for sisters. The difference in migration of their bands in the stacking gel was reproducible (see fig.2, lanes 6 and 7). The pattern of these glycoproteins was consistent for a given donor throughout and between her lactations. Representative data (fig.2) enable comparison of samples processed for day 16 and day 301 of one donor (lanes 3 and 2, respectively), and between two lactations for each of two donors (lane 1 vs lane 2 or 3 and lane 4 vs lane 5). In an earlier study [10], we observed that the HM_r glycoproteins are the strongest PAS-staining proteins of the human MFG.

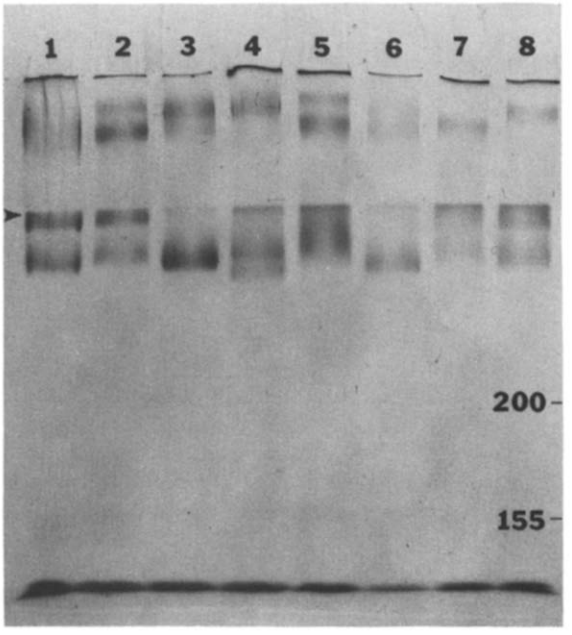

Fig. 1. SDS-polyacrylamide gel electrophoretogram showing HM_r glycoproteins from human MFGs. Samples contained 100 μg each of total globule proteins from 8 donors (lanes 1–8). Arrowhead (left) indicates junction of stacking and running gel. Staining was with PAS reagents. Positions of M_r references × 10^{-3} are shown on the right.

Frozen storage of globules, use of globules versus their SDS extracts or inclusion of protease inhibitors did not significantly alter the SDS-PAGE pattern of a sample. However, inclusion of globule fat tended at times to cause streaking and distortion of glycoprotein bands (not shown).

4. DISCUSSION

The polypeptide portion of the HM_r glycoproteins from human MFGs has not yet been characterized. It is therefore not known whether the differences between individuals in the patterns of these proteins (fig.1) are due to variations in structure of the peptides, the oligosaccharides, or both. However, the opportunity for structural diversity in the oligosaccharides is very great. There are many enzymes involved in the synthesis of these chains and each enzyme can have activity as well as a number of promoters and inhibitors unique to

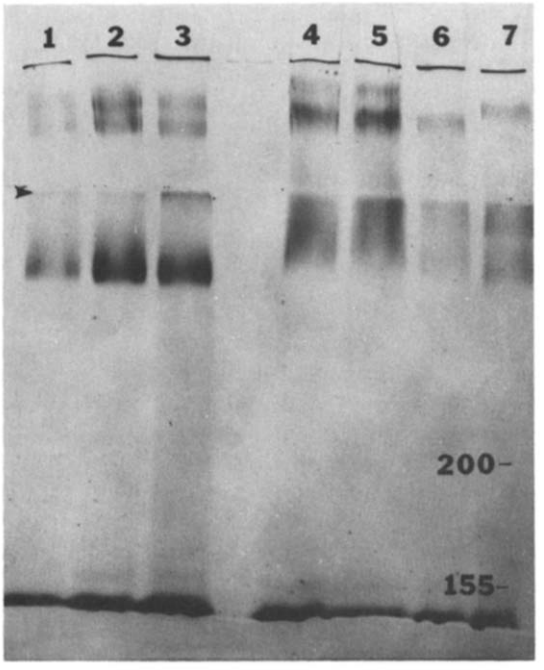

Fig.2. SDS-polyacrylamide gel electrophoretogram illustrating the effects of lactation time and kinship of donors on HM_r glycoprotein patterns of their MFGs. Lanes 1–3 are samples from the second lactation, third lactation-301 days and third lactation-16 days of a donor, respectively. Lanes 4 and 5 are samples from a second donor in her sixth and fifth lactations, respectively. Lanes 6 and 7 are samples from two other donors who are sisters. These are the same samples (from the same sisters) are shown in lanes 7 and 8, respectively, of fig.1. In comparing figs 1 and 2, note the reproducible difference in mobility of the slowest moving bands for the sisters' samples. Samples contained 100 μg of total globule proteins, excepting lane 1 (50 μg). Staining and other conditions were as for fig.1.

each individual [11,12]. Seven of the 8 samples used for the gel in fig.1 were chosen at random, the eighth was purposely sought to compare data for sisters. The patterns in fig.1 for this small number of donors clearly show individual diversity and those in fig.2 establish that it is a consistent trait.

HM_r glycoproteins have received limited attention. This is not surprising in that they are not resolved in SDS gels of suitable durability (>6% acrylamide) under conventional operating conditions, and they do not stain with Coomassie blue [3,10]. We view evidence of their human diversity in the MFG as a forerunner of what may be revealed in other tissues and cells. Fischer et al. [13] have reported that both peanut agglutinin and polyclonal antibodies to the HM_r glycoproteins of human MFGs bind to apical membranes of fundic glands in gastric mucosa, distal tubuli of human kidney, cells of histiocytic origin and some mammary carcinomas. A band of glycoprotein comigrating on SDS gels with those in our 4% gel (figs 1 and 2) has been detected in plasma membrane from porcine intestinal mucosal cells (Patton, S. and Patton, J.S., unpublished). Employing freeze-etch electron microscopy and SDS-PAGE, Buchheim et al. [10] have shown that the HM_r glycoproteins of the human MFG project from the globule approx. 0.5 μm as surface filaments. A similar glycocalyx has been revealed on absorptive cells of the cat intestine by freeze-etch electron microscopy [14]. Thus, it is possible that HM_r glycoproteins are common on many internal surfaces of the body.

Human MFG membrane has been shown to contain histocompatability antigens (HLA-DR) which were characterized as glycoproteins with 28 and 35 kDa components on denaturing gels [15]. We suggest that the HM_r glycoproteins may also be part of the immunologic recognition system because of their individual-specific nature and relatively large, extended structures on cell surfaces. In addition, they may provide a physical barrier protecting the cell.

ACKNOWLEDGEMENTS

We thank Dr J.S. O'Brien and J.S. Patton for discussion of our results. The University Hospital Milk Bank (Yvonne Vaucher, director) facilitated donations of human milk from area mothers. This research was supported by contract N01-HD6-2920 from the National Institutes of Child Health and Human Development.

REFERENCES

[1] Bargmann, W. and Knoop, A.-M. (1959) Z. Zellforsch. 49, 344–388.
[2] Patton, S. and Keenan, T.W. (1975) Biochim. Biophys. Acta 415, 273–309.
[3] Shimizu, M. and Yamauchi, K. (1982) J. Biochem. 91, 515–524.

[4] Shimizu, M., Yamauchi, K., Miyauchi, Y., Sakurai, T., Tokugawa, K. and McIllinney, R.A.J. (1986) Biochem. J. 233, 725–730.
[5] Patton, S. and Huston, G.E. (1986) Lipids 21, 170–174.
[6] Horowitz, W. (1975) Official Methods of Analysis of the AOAC, p. 258. Association of Official Analytical Chemists, Washington, DC.
[7] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[8] Laemmli, U.K. (1970) Nature 227, 680–685.
[9] Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2617.
[10] Buchheim, W., Welsch, U., Huston, G.E. and Patton, S. (1987) Pediatrics, in press.
[11] Beyer, T.A. and Hill, R.L. (1982) in: The Glycoconjugates (Horowitz, M.I. ed.) vol. 3, pp. 25–45, Academic Press, New York.
[12] Warner, T.G. and O'Brien, J.S. (1983) Annu. Rev. Genet. 17, 395–441.
[13] Fischer, J., Klein, P.-J., Farrar, G.H., Hanisch, F.-G. and Uhlenbruck, G. (1984) Biochem. J. 224, 581–589.
[14] Ito, S. (1974) Phil. Trans. R. Soc. London B268, 55–66.
[15] Wiman, K., Curman, B., Trägårdh, L. and Peterson, P.A. (1979) Eur. J. Immunol. 9, 190–195.