Vol. 132, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1985 Pages 299-306

MUCUS GLYCOPROTEIN FATTY ACYLTRANSFERASE IN PATIENTS WITH CYSTIC FIBROSIS: EFFECT ON THE GLYCOPROTEIN VISCOSITY

A. Slomiany, Y.H. Liau, S.R. Carter, L.J. Newman* and B.L. Slomiany

Gastroenterology Research Laboratory, Department of Medicine
and *Department of Pediatrics, New York Medical College, Valhalla, NY 10595

Received September 5, 1985

SUMMARY - The presence of an acyltransferase activity which catalyzes the transfer of palmitic acid from palmitoyl coenzyme A to mucus glycoprotein has been demonstrated in the microsomal fraction of human rectal mucosa. The activity of this enzyme in the mucosa of patients with cystic fibrosis (CF) was found to be 3.5 times higher than that from normal individuals. The CF mucus glycoprotein in comparison to that of normal contained 1.3 times more associated lipids and 6 times more covalently bound fatty acids. The viscosity of the intact CF glycoprotein was 1.8 times higher than that of normal glycoprotein. Extraction of associated lipids led to 3-fold drop in the viscosity of CF glycoprotein and 5-fold drop in the case of normal glycoprotein. Further loss in the viscosity occurred following removal of the covalently bound fatty acids. The viscosity of such modified CF mucus glycoprotein was only about 10% higher than that of similarly treated normal glycoprotein.

INTRODUCTION - Cystic fibrosis (CF), the common lethal disease of children and adults, results from a dysfunction of the exocrine glands (1,2). The abundant and abnormally thick mucus causes obstructions of the secretory ducts that impair the functions of various organs ultimately resulting in death. The nature of secretory abnormality in CF has never been established, although for years the abnormal appearance of mucus was attributed to the alteration in its glycoprotein constituent (2-4).

Recently, we have shown that alteration in CF mucus may be due to excessive acylation of mucus glycoprotein with fatty acids (5). The presence of an acyltransferase activity which catalyzes the transfer of fatty acid from acyl-CoA to mucus glycoprotein in gastric mucosa has been also demonstrated (6,7). Here, we report the presence and levels of an acyltransferase enzyme in rectal mucosa of normal and CF subjects, and show that the

0006-291X/85 $1.50

viscosity of mucus glycoprotein in CF is affected by the content of associated lipids and covalently bound fatty acids.

MATERIALS AND METHODS - The enzymatic studies were conducted on rectal mucosal biopsies obtained from 11 healthy subjects (age, 25-65 years) and 9 patients with CF (age, 2 months-17 years). The specimens, immediately following the endoscopy, were suspended in 5 vol of 3mM sodium phosphate buffer, pH 7.0, containing 0.25M sucrose and 1mM EDTA. The tissue was disintegrated with a glass-Teflon microhomogenizer, the homogenate was centrifuged at 800g for 10 min and the pellet discarded. The supernatant was centrifuged at 10,000g for 20 min to sediment the crude mitochondrial fraction and then separated into microsomal and cytosol fractions by centrifugation at 100,000g for 1h (6). The microsomal pellet was suspended in 0.25M buffered sucrose, pH 7.0, containing 0.5% Triton X-100, and the content of the tube was stirred at 4°C for 30 min. Following centrifugation at 100,000g for 1h, the resulting supernatant was used as an enzyme source.

The fatty acyltransferase assay mixtures contained the following components: acceptor glycoprotein, deacylated mucus glycoprotein 100µg; [1-^{14}C]-palmitoyl-CoA, 100µM (300,000cpm); Triton X-100, 0.5%; NaF, 20mM; dithiothreitol, 1.5mM; imidazole-HCl buffer at pH 7.4, 100mM; and enzyme protein 15-25µg, in a final volume of 100µl. The tubes were briefly sonicated and incubated at 37°C for 30 min. The reaction was terminated by immersing the tubes for 3 min in a boiling water bath and the mixture was centrifuged (6). The supernatant was spotted on Whatman 3MM paper strips (2.5 x 20cm), and the ^{14}C-labeled glycoprotein product was separated from the components of the reaction mixture by ascending chromatography (6). Following drying, the application zone was cut, placed in vials containing liquid scintillation solution and counted. The deacylated mucus glycoprotein acceptor was prepared from pig gastric mucus (8).

Gastric secretion, used for the isolation of mucus glycoprotein for viscosity measurements was obtained from 4 patients with CF and 5 healthy individuals (9).

Following dialysis and lyophilization, the dry residue was dissolved in 6M urea and chromatographed on a Bio-Gel A-50 column, equilibrated and eluted with 6M urea - 0.05M sodium phosphate buffer, pH 7.0. The eluate was monitored for protein and carbohydrate (8), and the fractions containing the excluded mucus glycoprotein peak were collected. Following rechromatography, the dialyzed and lyophilized high molecular weight mucus glycoprotein was dissolved in 0.05M phosphate buffer - 0.15M NaCl, pH 7.0, containing 42% (w/w) CsCl and subjected to equilibrium density gradient centrifugation (8). Delipidation of the purified normal and CF mucus glycoprotein preparation was accomplished by five consecutive extractions with chloroform-methanol (5). The extracts were filtered through Millipore FH (0.5µm) filters to retain the insoluble glycoprotein residue, and the lipids contained in the filtrates were recovered by evaporation. Removal of the covalently bound fatty acids from the delipidated glycoprotein was accomplished with hydroxylamine (5). Reassociation of the delipidated, and delipidated and deacylated glycoproteins with their lipids was performed in 0.10M NaCl - 0.05M phosphate buffer, pH 7.2, containing 0.02% NaN_3.

Viscosity determinations on various mucus glycoprotein preparations were performed with a Brookfield cone/plate digital viscometer equipped with a 1.565° cone and a constant (37°C) temperature bath (10). Shear rates were varied from 1.15 to 230s^{-1}, and the sample volumes were 0.5ml. For the measurements, preparations of normal and CF mucus glycoprotein were dissolved at 5mg/ml in 0.10M NaCl - 0.05M phosphate buffer, pH 6.0, containing 0.02% NaN_3, briefly sonicated and left for 16h at 4°C with gentle stirring (10). Prior to measurement, the samples were brought to 37°C. To calculate the specific viscosity (η_{sp}), the measurements were also taken of buffer alone.

The content and composition of carbohydrate in the purified glycoproteins was determined by gas-liquid chromatography (8). The protein was measured by the method of Lowry et al. (11), and the covalently bound fatty acids were quantitated as described in (5). The content of neutral lipids was determined according to procedures in (12), phospholipids by the method of Lowry and Tinlsey (13), and glycolipids by analysis of their carbohydrate component (12). Statistical analysis was performed using Student's t-test.

RESULTS - The distribution of fatty acyltransferase activity for the synthesis of acylated mucus glycoprotein in the subcellular fractions of normal human rectal mucosa is given in Table I. The enzyme activity for fatty acylation of mucin was located mainly in the microsomal fraction, the specific activity of which was about 11-fold higher than that of the crude homogenate. Optimum activity for acylation of mucus glycoprotein was obtained with palmitoyl-CoA in imidazole-HCl buffer at pH 7.4. The enzymatic transfer of palmitic acid to the deacylated glycoprotein was stimulated by Triton X-100, NaF and dithiothreitol, while $MgCl_2$ and EDTA were inhibitory (Table II). The rate palmitic acid transfer was proportional to microsomal enzyme protein concentration up to 100μg and remained constant with time of incubation for up to 1h. Addition of deacylated mucus glycoprotein to the incubation mixtures containing microsomal enzyme produced 3-fold stimualtion of the synthesis of ^{14}C-labeled glycoprotein. Treatment of the synthesized ^{14}C-labeled glycoprotein with methanolic KOH followed by hexane extraction (6), resulted in the liberation of the label from the glycoprotein to the hexane phase. This hexane extractable material co-chromatographed on thin-layer plates with the methyl palmitate standard and

Table I. Distribution of mucus glycoprotein fatty acyltransferase activity in subcellular fractions of human rectal mucosa

Fraction	Specific activity (nmol/mg protein·30 min)
Total homogenate	0.05 ± 0.01
Mitochondrial	0.13 ± 0.03
Microsomal	0.57 ± 0.20
Cytosol	0.02 ± 0.00

Enzymatic activity was measured using standard assay conditions described in the text. Values represent the means ± SD of five separate experiments performed in duplicate.

Table II. Requirements for human rectal mucosa mucus glycoprotein fatty acyltransferase activity

Incubation mixture	Relative % of specific activity
Complete	100
Minus acceptor mucin	31.6 ± 4.5
Minus Triton X-100	45.4 ± 4.1
Minus NaF	35.6 ± 5.3
Minus dithiothreitol	65.2 ± 5.8
Plus $MgCl_2$ (10mM)	78.3 ± 8.1
Plus EDTA (10mM)	82.1 ± 8.5

The composition of complete incubation mixture is given under Materials and Methods. The enzymatic activity was measured using standard assay conditions described in the text. Values represent the means ± SD of four separate experiments performed in duplicate.

accounted for 87% of the [^{14}C]palmitate incorporated to mucus glycoprotein in the enzyme assay systems.

The acylatransferase activity of the microsomal enzyme derived from rectal mucosal biopsies of normal indivduals and patients with CF is given in Table III. The results of assays indicated that CF samples consistently showed higher values for the activity of acyltransferase enzyme. On the average, the activity of this enzyme in rectal mucosa of CF patients was about 3.5 times higher ($P < 0.001$) than in normal subjects.

The chemical composition of the undegraded mucus glycoprotein purified from gastric secretion of healthy and CF individuals is given in Table IV. The CF mucus glycoprotein in comparison to that of normal, contained 1.3 times more associated lipids and about 6 times more covalently bound fatty acids. Lipids derived from each type of sample were of similar composition.

Table III. Activity of mucus glycoprotein fatty acyltransferase in rectal mucosa of normal individuals and patients with CF

Rectal mucosa	Number of individuals	Specific activity (nmol/mg protein·30 min)
Normal	11	0.52 ± 0.19
CF	9	1.78 ± 0.65*

Enzymatic activity of the microsomal fraction was measured using standard assay conditions described in the text. Values represent the means ± SD of duplicate analyses. *$P < 0.001$. The specific activity of normal samples ranged from 0.43 to 0.69, while that of CF ranged from 1.32 to 2.51.

Table IV. Chemical composition of human gastric mucus glycoprotein from normal and cystic fibrosis subjects

Constituent (mg/100mg)	Mucus glycoprotein	
	Normal	CF
Protein	13.0 ± 2.6	11.8 ± 2.1
Carbohydrate	61.3 ± 4.1	58.6 ± 6.2
Associated lipids		
Neutral lipids	11.1 ± 1.7	14.6 ± 1.5
Phospholipids	2.6 ± 0.3	3.4 ± 0.4
Glycolipids	6.5 ± 0.6	8.5 ± 0.7
Covalently bound fatty acids	0.3 ± 0.1	1.8 ± 0.3

Each value represents the means ± SD of triplicate analyses.

The covalently bound fatty acids consisted mainly (87%) of hexadecanoic and octadecanoic acids, whereas the associated lipids were represented by neutral lipids (55%), glycolipids (32%) and phospholipids (13).

The viscosity of intact gastric mucus glycoprotein from normal and CF subjects as a function of shear rate is shown in Figure 1. The data indicate that the viscosity of CF glycoprotein over the entire tested range was about 1.8 greater than that of normal glycoprotein. Extraction of associated lipids from the CF mucus glycoprotein caused about 3-fold drop in the viscosity, while the viscosity of normal glycoprotein decreased about 5-fold. The viscosity of the delipidated CF mucus glycoprotein, how-

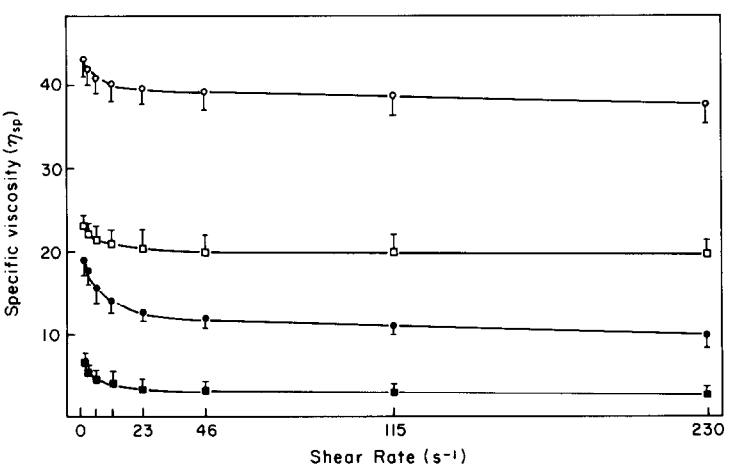

Fig. 1. Effect of associated lipids removal on the viscosity of gastric mucus glycoprotein from normal individuals and patients with CF. Intact CF glycoprotein (○); intact normal glycoprotein (□); delipidated CF glycoprotein (●); delipidated normal glycoprotein (■).

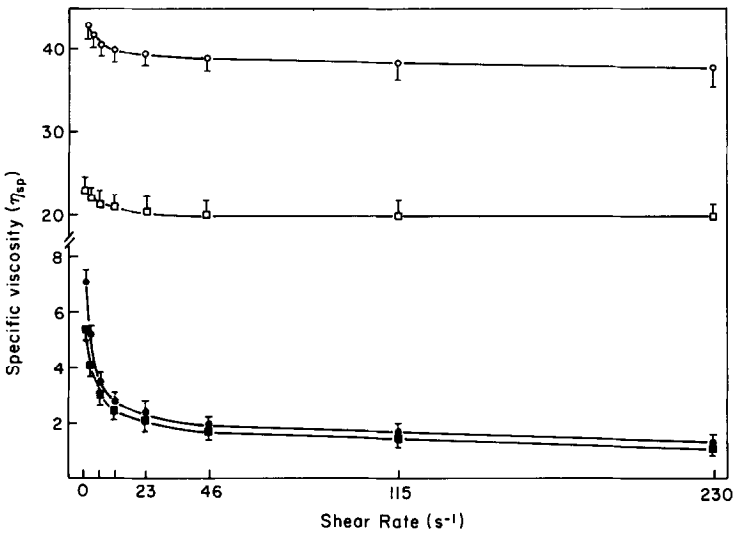

Fig. 2. Effect of delipidation and fatty acid deacylation on the viscosity of normal and CF gastric mucus glycoprotein. Intact CF glycoprotein (○); intact normal glycoprotein (□); delipidated and deacylated CF glycoprotein (●); delipidated and deacylated normal glycoprotein (■).

ever, still remained considerably higher (Fig. 1) than that of the delipidated normal glycoprotein. Further loss in viscosity of both glycoproteins occurred following their deacylation. Removal of the covalently bound fatty acid from the delipidated samples resulted in 1.5-fold drop in the viscosity of normal mucus glycoprotein and about 5-fold drop in the viscosity of CF glycoprotein (Fig. 2). The viscosity of such delipidated and deacylated CF mucus glycoprotein was only about 10% higher than that of normal glycoprotein.

DISCUSSION - Studies on mucus glycoproteins of saliva and gastrointestinal secretions indicate that these important constituents of mucus in addition to protein and carbohydrate also contain covalently bound fatty acids (5,6,14,15). The ester bound fatty acids apparently determine the extent of mucin susceptibility to proteolytic degradation, affect the viscosity, and influence its permeability to ions and other molecules (14,16-18). As the acylation of mucus glycoproteins with fatty acids is controlled by a specific acyltransferase, the occurrence of which was demonstrated in salivary glands and gastric mucosa of rat (6,19), we have investigated the

presence and activity of this enzyme in human mucosal tissue. The data obtained demonstrate that the acyltransferase enzyme also occurs in human rectal mucosa. Like in rat, the human rectal mucosa acyltransferase exhibits pH optimum of 7.4, is concentrated in the microsomal fraction and requires detergent, NaF, and dithiothreitol for its maximal activity. Employing optimal conditions for the assay of mucus glycoprotein fatty acyltransferase, it was found that the activity of this enzyme in the rectal mucosa from patients with CF was sigficantly ($P < 0.001$) higher than that from healthy individuals. The finding suggests that the excessive acylation of mucus glycoproteins with fatty acids in CF results from an increase in the acyltransferase activity.

Since gastric mucus glycoprotein from patients with CF exhibits higher content of lipids than that from normal indivdiual, we have also investigated how these lipids influence the viscosity of the glycoprotein. The results obtained show that while the extraction of associated lipids lead to a substantial drop in viscosity of both glycoproteins, the viscosity of the delipidated CF glycoprotein still remained substantially higher. However, removal of associated lipids and covalently bound fatty acids from normal and CF glycoproteins resulted in preparations which exhibited nearly identical viscosities. These findings indicate that although associated lipids contribute to the viscosity of mucus glycoproteins, it is the content of covalently bound fatty acids which is responsible for the abnormal viscosity of this glycoprotein in CF. Accordingly, the activity of acyltransferase enzyme appears to be a major factor responsible for the abnormally thick mucus in CF which causes obstruction and malfunction of the glandular ducts. Thus, it is rational to suggest that the measurements of the mucus glycoprotein fatty acyltransferase activity in mucosal biopsies can be used as a reliable test in the diagnosis of cystic fibrosis.

ACKNOWLEDGEMENTS - This work was supported by USPHS Grant HL# 32553-01 from the National Heart, Lung, and Blood Institute and Grant AM# 21684-08 from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, NIH.

REFERENCES

1. Davis, P.B., and Di Sant'Agnese, P.A. (1980) Pediatr. Res. 14, 83-87.
2. Boat, T.F., and Cheng, P.W. (1980) Fed. Proc. 39, 3067-3074.
3. Boat, T.F., and Matthews, L.W. (1973) in Sputum (Dulfano, M.F. ed.) pp. 267-269, C.C. Thores Publisher, Springfield, IL.
4. Forstner, J.F. (1978) Digestion 17, 234-263.
5. Slomiany, A., Witas, H., Aono, M., and Slomiany, B.L. (1983) J. Biol. Chem. 258, 8535-8538.
6. Slomiany, A., Liau, Y.H., Takagi, A., Laszewicz, W., and Slomiany, B.L. (1984) J. Biol. Chem. 259, 13304-13308.
7. Liau, Y.H., Slomiany, B.L., Slomiany, A., Piasek, A., Palmer, D., and Rosenthal, W.S. (1985) Digestion 32, 57-62.
8. Laszewicz, W., Slomiany, A., Murty, V.L.N., Liau, Y.H., and Slomiany, B.L. (1985) Digestion 31, 47-53.
9. Slomiany, A., Slomiany, B.L., Witas, H., Zdebska, E., Galicki, N.I., and Newman, L.J. (1983) Biochim. Biophys. Acta 750, 253-260.
10. Murty, V.L.N., Sarosiek, J., Slomiany, A., and Slomiany, B.L. (1984) Biochem. Biophys. Res. Commun. 121, 521-529.
11. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
12. Slomiany, B.L., Murty, V.L.N., Aono, M., Slomiany, A., and Mandel, I.D. (1982) Arch. Oral Biol. 27, 803-807.
13. Lowry, R.R. and Tinsley, I.J. (1974) Lipids 9, 491-492.
14. Slomiany, A., Jozwiak, Z., Takagi, A., and Slomiany, B.L. (1984) Arch. Biochem. Biophys. 229, 560-567.
15. Slomiany, B.L., Jozwiak, Z., Slomiany, A., Takagi, A., and Mandel, I.D. (1983) IRCS Med. Sci. 11, 765.
16. Sarosiek, J., Slomiany, A., Takagi, A., and Slomiany, B.L. (1984) Biochem. Biophys. Res. Commun. 118, 523-531.
17. Murty, V.L.N., Sarosiek, J., Slomiany, A., and Slomiany, B.L. (1984) Biochem. Biophys. Res. Commun. 121, 521-529.
18. Piasek, A., Slomiany, A., and Slomiany, B.L. (1985) Gastroenterology 88, 1539.
19. Liau, Y.H., Slomiany, A., and Slomiany, B.L. (1985) Fed. Proc. 44, 1642.