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PROSTAGLANDIN EFFECT ON THE ENZYMATIC SULFATION OF MUCUS GLYCOPROTEIN IN GASTRIC MUCOSA

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Abstract—The effect of 16,16-dimethyl prostaglandin E_2 ($DMPGE_2$) on the sulfation of mucus glycoprotein in gastric mucosa was investigated. The enzymatic activity which catalyzes the transfer of the sulfate ester group from 3′-phosphoadenosine-5′-phosphosulfate to gastric mucus glycoprotein was located in the detergent extracts of Golgi-rich membrane fraction of antral and body mucosa of rat stomach. The sulfotransferase activity of this fraction from body mucosa, however, was 35% higher than that from the antrum. The enzyme exhibited optimum activity at pH 6.8 using 0.5% Triton X-100 and 30 mM NaF. The apparent K_m of the enzyme for sulfation of mucus glycoprotein was 10.5 μM, and the sulfate ester was found incorporated into the carbohydrate chains of the glycoprotein. Introduction of $DMPGE_2$ to the reaction mixtures led to an enhancement in the rate of mucus glycoprotein sulfation. The rate of enhancement was proportional to the concentration of $DMPGE_2$ up to 1.0×10^{-4} M and was of the competitive type, with an apparent K_m value of 6.7 μM. Since sulfated mucins play an important role in gastric mucosal defense and the increase in their sulfation occurred at levels of prostaglandin present in gastric mucosa, the observed effect may be of significance to gastric mucosal defense *in vivo*.

The mucus layer covering the epithelial surfaces of gastric mucosa constitutes the first line of mucosal defense against a variety of noxious agents [1–4]. The integrity of this layer, essential for the protective function, is maintained through the interaction of its mucus glycoprotein, protein and lipids constituents, and is directly related to the rate of mucus synthesis, secretion, and breakdown [4–6]. Among the factors which control these events are prostaglandins. These compounds and many of their methyl analogs, including 16,16-dimethyl prostaglandin E_2 ($DMPGE_2$), have been shown to stimulate gastric mucus production and secretion leading to an increase in thickness of the adherent mucus coat [7–10]. The beneficiary effects of prostaglandins apparently result from their ability to control the processes involved in the synthesis of mucus glycoprotein, which constitutes the gel matrix [1, 4].

The process of this glycoprotein assembly proceeds through the stages of ribosomal synthesis of apomucin peptide core, its acylation with fatty acids, translocation into the lumen of endoplasmic reticulum, and the initiation of glycosylation [11, 12]. Further processing of mucus glycoprotein involves such post-translational modifications as elongation of carbohydrate chains, polymer assembly, and decoration of certain sugar units with sulfate ester groups [11, 13]. The sulfate groups occurring in gastrointestinal mucins in linkage with *N*-acetylglucosamine and galactose [14, 15] impart strong anionic character to mucus glycoprotein molecules and thus influence acid impedence and viscoelastic properties of mucus gel, modulate the corrosive action of pepsin in the stomach, and impart to the glycoprotein a high degree of inertness to proteolysis and action of glycosylhydrolazes [3, 11, 16]. Thus, the enzyme system responsible for mucus glycoprotein sulfation plays an important role in the maintenance of gastric mucus gel resilience and, therefore, mucosal resistance to injury. Yet, with the exception of studies on the incorporation of sulfate to mucus glycoproteins by the mucosa exposed to different adverse situations [17–19], nothing is known about the sulfotransferase involved in mucus glycoprotein sulfation and the effect of prostaglandins on the activity of this enzyme. Here, we report the effect of $DMPGE_2$ on the mucus sulfotransferase activity in gastric mucosa.

MATERIALS AND METHODS

Enzyme preparation. Male Sprague–Dawley rats (180–200 g), fasted overnight prior to experiment, were killed by decapitation, and their stomachs were removed, opened along the greater curvature, and washed with cold saline. The mucosa from antral and body areas was scraped with a glass slide, separately suspended in 5 vol. of 3 mM phosphate buffer, pH 7.0, containing 0.25 M sucrose and 1 mM EDTA, and homogenized in a glass–Teflon homogenizer [20]. The homogenate was centrifuged at 800 *g* for 10 min, the pellet was discarded, and the supernatant fraction was centrifuged at 10,000 *g* for 20 min to

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sediment the crude mitochondrial fraction. Centrifugation of the resulting supernatant at 100,000 *g* for 1 hr produced the microsomal (pellet) and cytosol fractions (supernatant). The Golgi-rich fraction of body and antral mucosa was isolated from the interface between 0.32 and 1.20 M sucrose layers [21]. The pellet fractions were solubilized by stirring at 4° for 30 min with 0.25 M buffered sucrose, pH 7.0, containing 0.5% Triton X-100, followed by centrifugation at 100,000 *g* for 1 hr. The solubilized enzyme preparations were stored at −20°. Protein content of the obtained fractions was determined by the method of Lowry *et al.* [22].

Mucus glycoprotein acceptor preparation. Gastric mucus glycoprotein polymer was prepared from mucus obtained by instillation of freshly dissected pig stomachs with buffered, pH 7.0, 2 M NaCl [23]. Following dialysis and lyophilization, the mucus was dissolved in 6 M urea and chromatographed on a Bio-Gel A-50 column, equilibrated in and eluted with 6 M urea–10 mM phosphate buffer, pH 7.0. Fractions containing the excluded mucus glycoprotein peak were pooled and subjected to equilibrium density gradient centrifugation in CsCl. For this, the lyophilized glycoprotein was dissolved in 0.05 M phosphate buffer–0.15 M NaCl, pH 7.0, containing 42% (w/w) CsCl and centrifuged for 48 hr at 12° and 46,000 rpm in a Beckman 50Ti rotor. The mucus glycoprotein, recovered from the density gradient tubes with the aid of a Beckman recovery system, was dialyzed against distilled water and lyophilized.

Removal of the sulfate ester groups from the isolated mucus glycoprotein was accomplished by acid catalyzed solvolysis [24]. The glycoprotein was sonicated with 0.05 M HCl in dry methanol at 32° for 4 hr, and following neutralization the mixture was dialyzed against distilled water and lyophilized. The sulfate content of the intact and desulfated mucus glycoprotein preparations was determined turbidimetrically [25, 26], and the content and composition of carbohydrates were determined by gas–liquid chromatography following methanolysis, re-N-acetylation and derivatization with silylating reagent [27]. Covalently bound fatty acids were analyzed by gas–liquid chromatography as their methyl esters [5].

Incubation system. The reaction mixtures for mucus glycoprotein sulfotransferase assays, incubated at 37° in a total volume of 100 μl, consisted of 50–800 μg desulfated gastric mucus glycoprotein, 0.5 to 15 μM 3′-phosphoadenosine-5′-phospho[^{35}S]sulfate (PAPS), 0.5% Triton X-100, 30 mM NaF, 0 to 1.0×10^3 M $DMPGE_2$, enzyme fraction containing 20–60 μg protein, and 100 mM imidazole–HCl buffer, pH 6.8. The tubes containing the complete incubation mixtures were briefly sonicated, and the reaction was initiated by the addition of enzyme preparation. In the initial experiments, the reaction mixtures were incubated in a shaking water bath at 37° for various periods of time up to 2 hr. In the standard assays, the reaction was terminated after 45 min by addition of 0.3 ml of ethanol. The resulting mixture was kept overnight at 4° and the formed precipitate was collected by centrifugation [26]. The pellet was dissolved in 0.1 ml of water, and the ethanol precipation and centrifugation procedure was repeated two more times. The final sediment was dissolved in 0.2 ml of 0.1 M citrate buffer, pH 3.6, and the ^{35}S-glycoprotein product was separated from the components of the reaction mixtures by chromatography on a Bio-Gel P-30 column (0.7 × 10 cm). The ^{35}S-labeled glycoprotein, eluted in the excluded volume of this column, was then placed in vials containing scintillation solution and counted in a scintillation counter.

The enzyme activity was expressed as picomoles of [^{35}S]sulfate ester group transferred to mucus glycoprotein acceptor per mg of enzyme protein per 45 min. All experiments were carried out in the presence of controls that contained the incubation mixtures minus glycoprotein acceptor or boiled enzyme. Assays were run in duplicate, and the results are expressed as means ± SD. Student's *t*-test was used to test significance, and P values of 0.05 or less were considered as significant.

^{35}S-Glycoprotein product identification. For the identification of ^{35}S-glycoprotein product of enzymic reaction, the incubates from twenty-eight assay tubes were combined, precipitated with ethanol, and subjected to gel filtration on a Bio-Gel P-30 column (1.5 × 90 cm), equilibrated in and eluted with 0.1 M citrate buffer, pH 3.6. The ^{35}S-glycoprotein, recovered in the excluded volume, was dialyzed against distilled water, lyophilized and subjected to equilibrium density gradient centrifugation in CsCl. Fractions of 1 ml were collected, and each fraction was assayed for protein and carbohydrate, and screened for radioactivity by scintillation spectrometry.

Reductive cleavage of the carbohydrate chains of synthesized ^{35}S-glycoprotein was performed with alkaline borohydride using 0.05 M KOH in 1.0 M $NaBH_4$ [27]. Following incubation at 45° for 16 hr, the reaction mixture was cooled to 4°, the excess of borohydride was destroyed by titration to pH 5.0 with diluted acetic acid, and the mixture was freed of boric acid by co-distillation with methanol. The liberated reduced oligosaccharides were then separated into neutral and acidic fractions by chromatography on a AGl-X2 (Cl^-) column [27]. The recovered oligosaccharide fractions were desalted on a Sephadex G-10 column (0.9 × 110 cm), concentrated in a rotary evaporator, and chromatographed on thin-layer plates in 1-butanol–acetic acid–water (3:3:2, by vol.). Following chromatography, the plates were scanned for ^{35}S-label using the Berthold Linear Analyzer System.

RESULTS

The chemical characteristics of the undegraded mucus glycoprotein purified from pig gastric mucus are given in Table 1. The glycoprotein contained 14.3% protein, 61.7% carbohydrate, 2.8% sulfate, and 0.4% covalently bound fatty acids. This glycoprotein was used for preparation of substrate for the assay of gastric mucosal mucus glycoprotein sulfotransferase. Acid catalyzed solvolysis of the glycoprotein followed by chemical analysis of the product revealed that the sulfate content of the prepared glycoprotein acceptor decreased to 0.2%, while the

Table 1. Chemical composition of the intact and desulfated gastric mucus glycoprotein used for the assay of the gastric mucosal sulfotransferase activity

Component	Relative weight (mg/100 mg)	
	Intact	Desulfated
Fucose	9.7 ± 1.1	9.5 ± 1.0
Galactose	21.6 ± 2.4	22.0 ± 2.3
N-Acetylgalactosamine	13.1 ± 1.2	13.5 ± 1.4
N-Acetylglucosamine	16.4 ± 1.7	16.9 ± 1.8
Sialic acid	0.9 ± 0.1	0.7 ± 0.2
Sulfate	2.8 ± 1.5	0.2 ± 0.1
Protein	14.3 ± 1.5	14.8 ± 1.5
Covalently bound fatty acids	0.4 ± 0.2	0.2 ± 0.1

Values represent the means ± SD of triplicate analyses performed on each sample.

Table 2. Mucus glycoprotein sulfotransferase activity in subcellular fraction of rat gastric mucosa

Fraction	Specific activity (pmol/mg protein·45 min)	
	Body	Antrum
Total homogenate	3.21 ± 0.35	1.73 ± 0.15
Mitochondrial	0.71 ± 0.09	0.40 ± 0.06
Microsomal	5.90 ± 0.61	4.11 ± 0.48
Golgi-rich	18.03 ± 1.94	13.32 ± 1.39
Cytosol	0.41 ± 0.05	0.36 ± 0.07

Incubation mixtures contained the following components in a final volume of 100 μl: 300 μg desulfated gastric mucus glycoprotein, 6 μM [^{35}S]PAPS, enzyme fraction containing 20–60 μg protein, 0.5% Triton X-100, 30 mM NaF, and 100 mM imidazole–HCl buffer, pH 6.8. Each value represents the means ± SD of four separate experiments performed in duplicate.

content and composition of protein and carbohydrate remained quite similar to that of the intact glycoprotein (Table 1).

Analysis of the distribution of sulfotransferase activity for the synthesis of sulfated mucus glycoprotein in the subcellular fractions of body and antrum mucosa is presented in Table 2. In both areas of the stomach, the enzyme activity was located mainly in the microsomal fraction, and further subcellular fractionation revealed that the enzyme is associated with the Golgi-rich membrane fraction. Compared to crude homogenate, the specific activity of this fraction from antral mucosa was enriched 7.7-fold, while 6-fold enrichment in the sulfotransferase activity was obtained with the body mucosa. The specific activity of the sulfotransferase solubilized from Golgi-rich fraction of body mucosa was, however, 35% higher than that from antrum. Therefore, the Golgi-rich fraction from body mucosa was used as the sulfotransferase source for further experiments.

Studies on the requirements of body mucosal mucus glycoprotein sulfotransferase enzyme established that the maximum activity for transfer of the sulfate ester group from PAPS to the desulfated gastric mucus glycoprotein was at pH 6.8. The transferase activity was stimulated by Triton X-100 and NaF, whereas ATP, $MnCl_2$ and $MgCl_2$ were inhibitory at the concentrations examined (Table 3). The maximal sulfotransferase activity was obtained with 0.5% Triton X-100 and 30 mM NaF. Under optimal conditions, the rate of sulfate ester group transfer to mucus glycoprotein acceptor was proportional to Golgi enzyme protein concentration up to 50 μg and remained constant with time of incubation for up to 90 min. There was no incorporation of sulfate to mucin acceptor in the absence of enzyme or with boiled enzyme. The enzyme effectively utilized as acceptor for sulfate from PAPS the desulfated gastric mucus glycoprotein, whereas the acceptor capacity of the intact glycoprotein was 68% lower (Table 3). The transfer of sulfate ester group to the desulfated mucus glycoprotein was proportionally enhanced, over a given range, with increasing concentrations of both substrates, PAPS and the glycoprotein. The effect of mucus glycoprotein concentration is illustrated in Fig. 1A and that of PAPS in Fig. 1B. The

Table 3. Requirements for rat stomach body mucosal mucus glycoprotein sulfotransferase activity

Incubation mixture	Specific activity (pmol/mg protein·45 min)
Complete	18.21 ± 1.86
Minus acceptor glycoprotein	2.01 ± 0.17
Minus Triton X-100	5.74 ± 0.46
Minus desulfated glycoprotein plus intact mucin	7.01 ± 0.83
Plus $MgCl_2$ (4 mM)	13.30 ± 1.36
Plus $MnCl_2$ (4 mM)	10.07 ± 1.10
Plus ATP (4 mM)	12.51 ± 1.18
Minus enzyme	0.07 ± 0.02
Minus active enzyme plus heat-inactivated enzyme	0.09 ± 0.03

The complete incubation mixture contained in 100 μl: 200 μg of desulfated mucus glycoprotein, 7.4 μM [^{35}S]PAPS, 20 μg of enzyme protein, 0.5% Triton X-100, 30 mM NaF, and 100 mM imidazole–HCl buffer, pH 6.8. Values represent the means ± SD of five separate experiments performed in duplicate.

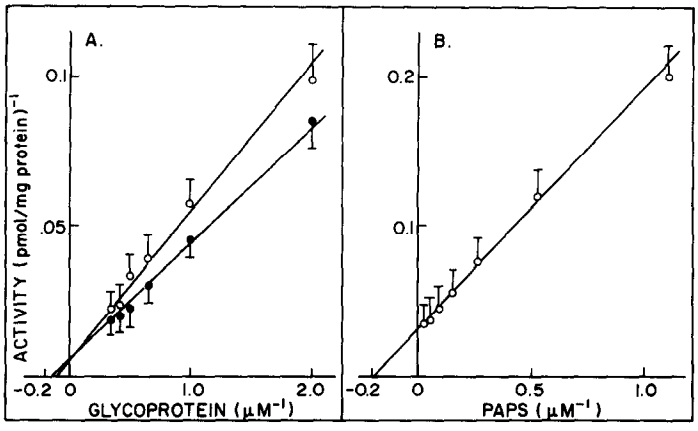

Fig. 1. (A) Effect of glycoprotein concentration on gastric mucosal mucus glycoprotein sulfotransferase activity in the absence (○) and presence (●) of 2.6×10^{-6} M 16,16-dimethyl prostaglandin E_2 ($DMPGE_2$). The composition of the incubation mixtures was the same as in Table 3, except that varied concentrations (50–800 μg) of desulfated glycoprotein substrate were used. (B) Effect of [^{35}S]PAPS concentration on gastric mucosal mucus glycoprotein sulfotransferase activity. The composition of the incubation mixtures was the same as in Table 3, except that varied concentrations (0.5 to 15 μM) of [^{35}S]PAPS were used. The data shown in each panel represent the means ± SD of four experiments performed in duplicate. The change in K_m value due to $DMPGE_2$ was significant at $P < 0.001$.

apparent K_m value for gastric mucus glycoprotein, based on the molecular weight of 1.0×10^6 [1, 7], calculated from Lineweaver–Burk plots was 10.5 μM. The apparent K_m for PAPS with body mucosal enzyme was 5.1 μM (Fig. 1).

The ^{35}S-labeled mucus glycoprotein, synthesized in the presence of body mucosa sulfotransferase enzyme, gave on equilibrium density gradient centrifugation a band at the CsCl density of 1.46 in which the carbohydrate peak coincided with that of the ^{35}S-label. Treatment of this glycoprotein with alkaline borohydride resulted in the liberation of ^{35}S-label to the reduced oligosaccharide fraction. Following fractionation of the released oligosaccharides on a AG1-X2(Cl^-) column, 98.7% of the ^{35}S-label was found in the acidic oligosaccharide fraction. Thin-layer chromatography of this oligosaccharide fraction revealed the presence of three ^{35}S-labeled oligosaccharides which together accounted for 73% of the ^{35}S-glycoprotein label. The radioscan of the thin-layer chromatogram of the acidic oligosaccharides obtained from ^{35}S-glycoprotein synthesized *in vitro* in the presence of gastric mucosal enzyme is shown in Fig. 2.

The effect of prostaglandin concentration on the activity of gastric mucosal mucus glycoprotein sulfotransferase is illustrated in Fig. 3. The data presented show that the introduction of $DMPGE_2$ to the incubation mixtures caused an increase in the rate of mucus glycoprotein sulfation. The rate of

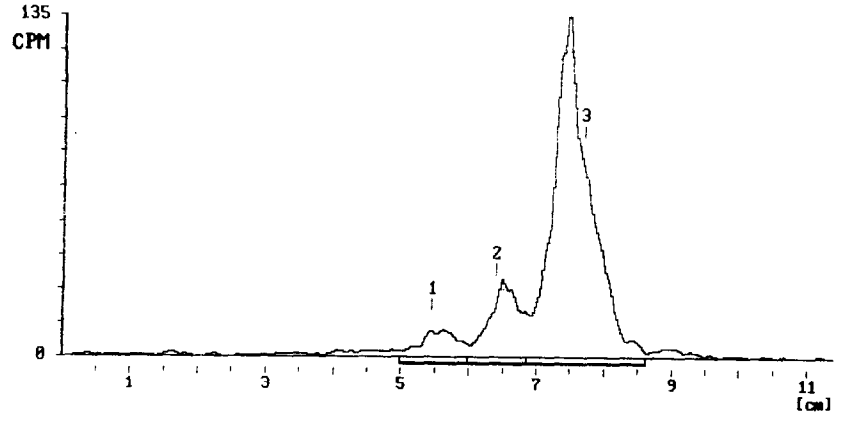

Fig. 2. Radioscan of thin-layer chromatography plate of the acidic oligosaccharides obtained from ^{35}S-glycoprotein, synthesized *in vitro* in the presence of gastric mucosal mucus glycoprotein sulfotransferase. Following chromatography in 1-butanol–acetic acid–water (3:3:2, by vol.), the plate was scanned for ^{35}S-label using the Berthold Linear Analyzer System. The positions of the labeled oligosaccharides are indicated by numbers.

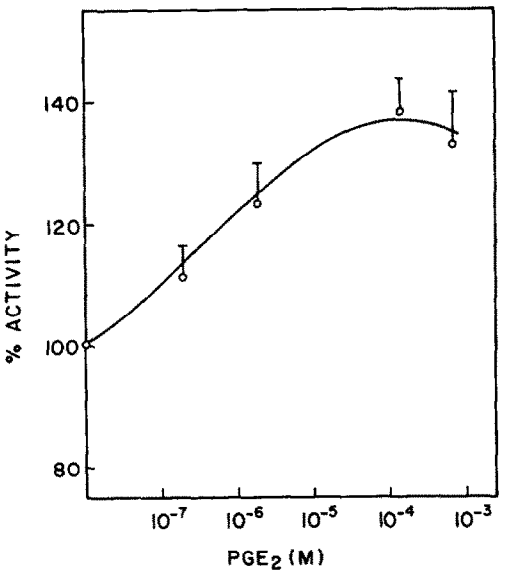

Fig. 3. Effect of $DMPGE_2$ concentration on the activity of gastric mucosal mucus glycoprotein sulfotransferase. The assay conditions were the same as described in Table 3, except that varied concentrations (0 to 1.0×10^3 M) of prostaglandin were included. The data show the means ± SD of five experiments performed in duplicate. The increase in the rate of mucus glycoprotein sulfation by 1.0×10^{-4} M prostaglandin was significant at $P < 0.001$.

enhancement of the glycoprotein sulfation by $DMPGE_2$ remained constant with time of incubation for 1 hr and was proportional to the drug concentration up to 1.0×10^{-4} M, at which concentration a 37% increase in mucus glycoprotein sulfation was attained. The apparent K_m for sulfation of gastric mucus glycoprotein in the presence of $DMPGE_2$ was 6.7 μM (Fig. 1A). Results of experiments in which mucus glycoprotein was preincubated for various periods of time up to 1 hr with $DMPGE_2$ prior to addition of the sulfotransferase enzyme indicated no stimulatory effect on the enzyme activity. However, when $DMPGE_2$ was preincubated with the enzyme before the addition of mucus glycoprotein substrate, an increase in the sulfotransferase activity was observed even after 1 min of preincubation.

DISCUSSION

The role of prostaglandin in the synthesis and secretion of mucus glycoproteins in gastrointestinal tract is recognized as an important factor for the maintenance of mucosal integrity, and the mechanism of prostaglandin-induced mucosal protection against damage by a variety of noxious agents is an area of extensive investigation [4, 8, 9, 28–30]. The majority of studies, however, assess the action of prostaglandins for the absence or reduction in the macroscopically visible necrotic lesions, or on the dynamic processes in the cells by light scanning and transmission microscopy [8, 9, 30–33]. The data on the effect of prostaglandins on the biosynthetic events leading to mucus glycoprotein elaboration are scant and limited to the measurements of mucin synthesis by following the radiolabeled sugar incorporation [28, 34]. The results of these types of studies demonstrated that prostaglandins and their stable methyl analogs stimulate the incorporation of fucose and glucosamine into gastric mucus glycoprotein, whereas the blockers of prostaglandin synthesis suppress mucus glycoprotein synthesis [8, 11, 34, 35]. Even less is known of the effect of prostaglandins on the enzyme systems that control mucus glycoprotein processing and the qualities of the elaborated product. One of such systems is an enzyme involved in the sulfation of mucus glycoprotein. Since sulfated mucins are implicated prominently in gastric mucosal defense, and changes in their synthesis signal the onset of various gastrointestinal diseases [17, 19, 36], we have investigated the effect of prostaglandin on the transferase involved in the synthesis of sulfated mucins in gastric mucosa.

The results of this study conducted with stable prostaglandin analog show that $DMPGE_2$ exerted a stimulatory effect on the activity of a sulfotransferase enzyme that catalyzes the transfer of the sulfate ester group from PAPS to mucus glycoprotein in gastric mucosa. Our data demonstrated that the rate of enhancement of mucin sulfation by this Golgi enzyme was proportional to $DMPGE_2$ concentration up to 1.0×10^{-4} M, at which point a 37% increase in sulfation occurred. Preincubation experiments showed that the enhancement in sulfotransferase activity took place rapidly and was due to interaction of prostaglandin with the enzyme and not with the glycoprotein substrate. As the increase in rate of mucin sulfation occurred at prostaglandin levels corresponding to that present in gastric mucosa which in humans is about 1.3×10^{-6} M [37], the observed effect may be of physiological importance. Interestingly, the concentration range of $DMPGE_2$ at which the enhancement in mucus glycoprotein sulfotransferase activity was observed is also well below the doses (25–200 μg) of other synthetic prostaglandin analogs used in gastric ulcer therapy such as misoprostil, enprostil or arbaprostil [38]. Consequently, a decrease in mucus glycoprotein sulfation observed with gastric disease may be a result of diminished mucosal synthesis of prostaglandin. This is supported by the findings that nonsteroidal anti-inflamatory agents, such as indomethacin and aspirin, which inhibit prostaglandin generation are also potent inhibitors of mucin sulfation [8, 26, 28].

The evidence is thus emerging that points towards the interdependence of gastric mucus glycoprotein sulfation on the mucosal capacity for prostaglandin generation. Prostaglandins have also been shown to enhance fatty acylation of gastric mucin and the extent of this glycoprotein interaction with lipids in mucus gel [4, 29, 39], and hence are directly involved in determining the protective qualities of mucus coat which constitutes the luminal perimeter of gastric mucosal defense. Whether prostaglandins exert a similar stimulatory effect on the enzyme systems involved in the biosynthesis of glycoproteins of gastric epithelium, which provides a second line of mucosal defense, remains to be established.

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