[Chem. Pharm. Bull. 33(5)2061—2068(1985)]

Studies on Biological Activities of Melanin from Marine Animals. IV. Influence of Fr. SM II (Squid Melanin) on a High Molecular Glycoprotein (Peak I) Level in Rat Gastric Mucosa, and Properties of Peak I as a Gastric Mucosal Defensive Factor

TSUTOMU MIMURA,* KAZUHIRO MAEDA, YASUO ODA, TAICHIROU TERADA, KEIKO YOSHIDA and SHIGERU AONUMA

Faculty of Pharmaceutical Sciences, Osaka University, Yamadaoka 1–6, Suita, Osaka 565, Japan

(Received August 2, 1984)

A low molecular weight melanoprotein from *Ommastrephes bartrami* LESUEL (Fr. SM II) has been reported to counteract the ulcer-inducing activities of phenylbutazone and aspirin.

Glycoproteins extracted from the stomach were fractionated on a Sepharose 6B column and three peaks were obtained (peaks I, II and III). The amount of peak I was decreased following the onset of ulceration caused by phenylbutazone and aspirin, and was increased by a concomitant administration of Fr. SM II.

The amount of lipid peroxide was found to reflect the degree of ulceration. The effect of peak I on lipid peroxidation was studied by incubating peak I with hepatic microsomes, arachidonic acid and gastric mucosa homogenate *in vitro*. The formation of lipid peroxide was inhibited by the addition of peak I. It was also demonstrated that peak I has a lysosomal membrane-stabilizing activity.

These results suggest that Fr. SM II manifests its anti-ulcerogenic activity by increasing peak I.

Keywords——squid melanin; anti-ulcerogenic activity; phenylbutazone ulcer; aspirin ulcer; gastric mucosa; lipid peroxidation

The authors have been studying the anti-ulcerogenic activity of low molecular weight melanoprotein (Fr. SM II) from ink bags of *Ommastrephes bartrami* LESUEL, and reported its gastric secretion inhibitory activity.[1,2] A strong relationship between the ulcer index and the amount of extractable glycoproteins in the mucous layer and gastric mucosa was noted in our previous paper, using phenylbutazone- and aspirin-induced ulcer models.[2] (The mucous layer and gastric mucosa are taken together and designated as the gastric glandular portion hereafter.) A similar result was also reported by Murakami *et al.*[3] for aspirin-induced ulceration. These results suggested that glycoproteins might have an important role in the gastric defensive mechanisms.

In the present work, the glycoproteins extracted from the gastric glandular portion were fractionated by gel filtration and the peaks obtained from normal and ulcerated stomachs were compared. The peak which appeared to be most closely related to the ulceration (specifically reduced in the ulcerated stomach, while the other peaks were not influenced) was studied to determine its effect on lipid peroxidation and on the lysosomal membrane.

Experimental

Fr. SM II——Fr. SM II was obtained from the ink bags of *Ommastrephes bartrami* LESUEL as described in a

previous paper.[1]

Experimental Ulcer Models in Rats——i) Phenylbutazone-Induced Gastric Ulcer: A phenylbutazone ulcer model was induced according to the method of Suzuki *et al.*[4] Male Wistar strain rats weighing about 200 g were fasted for 24 h, but were provided with water *ad libitum*. Each rat received 200 mg/kg of phenylbutazone (Sigma Chemical Co.) suspended in 5% Gum Arabic solution perorally. Rats were sacrificed at 3, 5 and 7 h after the administration of phenylbutazone and their stomachs were removed to examine the lesions in the glandular stomach area. The sum of the diameter of each lesion was used as an ulcer index. A test sample was administered intraperitoneally before the administration of phenylbutazone.

ii) Aspirin-Induced Gastric Ulcer: Male Wistar rats weighing about 200 g were deprived of food for 24 h. According to the method of Okabe *et al.*,[5] rats orally received 100 mg/kg of aspirin suspended in 5% Gum Arabic solution immediately after pylorus ligation. A test sample was administered intraperitoneally concurrently with the pylorus ligation. Five, seven and nine hours after the pylorus ligation, rats were sacrificed and their stomachs were removed to examine the lesions in the glandular stomach. The sum of the length of all lesions was used as an ulcer index.

Extraction of Glycoprotein from Gastric Glandular Portion——Glycoproteins were extracted from the gastric glandular portion according to the method described by Azuumi *et al.*[5] The stomachs removed from the rats were incised at the greater curvature, and rinsed lightly with phosphate-buffered saline (PBS) containing 0.01% NaN_3. The gastric glandular portion was scraped off and homogenized with 0.5 ml of 50 mM Tris–HCl buffer (pH 7.4) containing 2% Triton X-100. In order to complete the solubilization, the homogenate was incubated for 1 h at 37 °C. Then, the homogenate was centrifuged (8000 *g*) for 30 min at 4 °C. The precipitate was again subjected to the extraction procedure, and the first and the second supernatants were combined. The extract was applied to a Sepharose 6B column (1 cm i.d. × 70 cm) and eluted with Tris–HCl buffer (pH 7.4) containing 2% Triton X-100 (fractions of 1.6 ml/tube).

Glycoproteins were detected by the phenol–H_2SO_4 method.[6] Protein content was measured by using the Bio-Rad protein assay kit (Bio-Rad Chemical Ind., Co.). The detected peaks were then applied to a Bio-Beads SM-2 (Bio-Rad Chemical Ind., Co.) column (0.9 cm i.d. × 30 cm; elution buffer, 10 mM potassium phosphate pH 7.4) to remove Triton X-100 according to the method described by Holloway.[7] The peaks were lyophilized after dialysis and used for the following experiments.

Chemical Analysis of Glycoproteins——i) Determination of Hexosamine: Hexosamine was measured according to the method reported by Hiroi *et al.*[8] A precisely weighed sample was dissolved in 3 ml of papain solution (Difco: 50 units of papain in 100 ml of 0.2 M acetate buffer at pH 5.6 containing 2 mM ethylenediaminetetraacetic acid (EDTA), 4 mM cysteine and 0.88% NaCl) and digested for 20 h at 37 °C. Undigested components were eliminated by centrifugation (3000 rpm × 10 min). An aliquot of the supernatant was hydrolyzed in 2 N HCl in a sealed ampoule at 110 °C for 14 h. Hexosamine was determined by the colorimetric method of Gunner[9] using glucosamine hydrochloride (Nakarai Chemical Co.) as a standard.

ii) Determination of Sialic Acid: After hydrolysis of the sample for 1 h in 0.1 N H_2SO_4 at 80 °C, sialic acid was determined by using the thiobarbituric acid method,[10] with *N*-acetylneuraminic acid (Sigma Chemical Co.) as a standard.

iii) Determination of Uronic Acid: The sample was digested for 48 h at 37 °C in papain solution and allowed to stand overnight at 4 °C after the addition of 1% cetylpyridinium chloride solution. After centrifugation, the precipitate was re-dissolved in 0.01 N NaOH and reacted with carbazole according to the method of Bitter and Muir.[11] Glucuronic acid (Wako Pure Chemical Ind., Ltd.) was used as a standard.

Measurement of Lipid Peroxide *in Vivo*——Animals were bled from the carotid artery under ether anesthesia. The liver was perfused with 10 mM Tris–HCl buffer (pH 7.4) containing 150 mM KCl (KTB) from the portal vein, excised and homogenized (10% w/v) in cold KTB in a Teflon homogenizer. The stomach was opened and rinsed well with KTB. The gastric glandular portion was scraped off and homogenized. The homogenates were used immediately for measurement of lipid peroxide.

Lipid peroxide in the test sample was measured according to the method described by Ohkawa *et al* [12] To 0.2 ml of the homogenate, 8.1% sodium dodecyl sulfate (SDS, 0.2 ml), 20% acetate buffer (1.5 ml) and 0.8% thiobarbituric acid (TBA, 1.5 ml) were added, and the volume was brought up to 4 ml with distilled water. The reaction mixture was heated in boiling water for 1 h, then allowed to cool. Distilled water (1 ml) and *n*-butanol–pyridine (15 : 1) mixture (5 ml) were added to the test tube and mixed thoroughly. The chromophore formed by TBA was extracted into the *n*-butanol–pyridine layer after centrifugation (3000 rpm × 15 min). The absorbance was measured at 532 nm.

Lipid peroxide in serum was measured according to the method of Yagi *et al.*[13] Briefly, the lipids containing lipid peroxide were precipitated with protein by adding phosphotungstic acid in the presence of H_2SO_4 and the precipitates were reacted with TBA.

Lipid Peroxidation Test *in Vitro*——i) Preparation of Hepatic Microsome Suspension: According to the method described by Sato *et al.*,[14] the liver was excised from male Wistar rats weighing about 200 g. After perfusion with cold KTB, the liver was homogenized in KTB (20% w/v) in a Teflon homogenizer. The homogenate was centrifuged at 9000 *g* for 20 min. The supernatant was centrifuged again at 105000 *g* for 60 min and the precipitate was used as the

microsomal fraction.

ii) Preparation of Gastric Mucosa Homogenate: Male Wistar rats weighing about 200 g were fasted for 24 h and sacrificed. The stomach was removed and rinsed with KTB, and the mucous layer of the gastric glandular portion was rubbed off. The substratum of the gastric mucosa was scraped off, and homogenized in a Teflon homogenizer.

iii) Preparation of Arachidonic Acid: Sodium arachidonic acid (Sigma Chemical Co.) was dissolved in KTB to make 100 μg/ml, and kept in a tightly closed vial under N_2 gas. Each of the hepatic microsomal fraction, gastric mucosa homogenate and arachidonic acid solution was mixed with a sample dissolved in KTB, and incubated for 30 min at 37 °C. Lipid peroxide was measured by the SDS–TBA method as described before.

Preparation of Hepatic Lysosomes and the Measurement of Enzyme Activity Released from Lysosomes——According to the method reported by Weissman,[15] male Wistar rats weighing about 200 g were sacrificed and the liver was excised. After homogenization in 10 mM Tris–acetate buffer (pH 7.4) containing 0.25 M sucrose (STB) in a Teflon homogenizer, the homogenate was centrifuged at 800 *g* for 10 min. The supernatant was centrifuged again at 20000 *g* for 20 min. The precipitate was resuspended in STB and centrifuged once more at 20000 *g* for 20 min. The lysosomal fraction was obtained as a precipitate.

A sample was dissolved in STB and mixed with lysosomes. After a 30 min incubation at 37 °C, the mixture was centrifuged at 20000 *g* for 20 min. To examine the direct effect of a sample on the enzyme activity, the lysosomal fraction was rinsed with 0.01% Triton X-100 and the *N*-acetyl-β-glucosaminidase activity was measured after incubation with the sample.[16]

Statistical Analysis——Student's *t*-test was applied to assess the significance of differences between the mean values of the control group and the sample-administered groups.

Results

Change in the Elution Profiles of Glycoproteins Extracted from Stomachs of Variously Treated Rats on a Sepharose 6B Column

Animals were sacrificed 5 h after the administration of phenylbutazone and the stomachs were excised. Glycoproteins extracted from the gastric glandular portion in the presence of Triton X-100 were passed through a Sepharose 6B column. An aliquot from each fraction was subjected to the phenol–H_2SO_4 reaction and checked for protein with a protein assay kit.

As shown in Fig. 1, the height of peak I was apparently lowered by the phenylbutazone treatment, while the other two peaks remained unchanged. A similar result was observed in aspirin-induced gastric ulcer (not shown).

The hexose content in each peak was measured and compared among groups of rats given: i) nothing, ii) Fr. SM II, iii) phenylbutazone, iv) phenylbutazone and Fr. SM II, v) aspirin and vi) aspirin and Fr. SM II.

As shown in Table I, the administration of phenylbutazone or aspirin caused a significant loss of hexose content (40 and 31%, respectively) in peak I, while peaks II and III were not

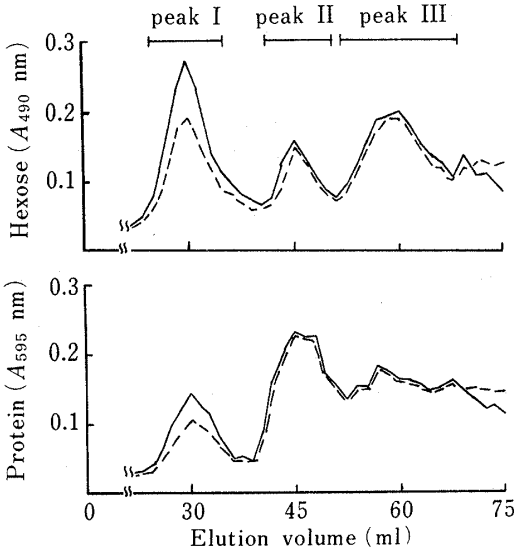

Fig. 1. Sepharose 6B Column Chromatography of Extract from the Gastric Glandular Portion of Rats with Phenylbutazone-Induced Ulceration

Animals were sacrificed 5 h after the administration of phenylbutazone and the stomachs were excised. Samples were analyzed by the phenol–H_2SO_4 method at A_{490} (upper panel) and by the Bio-Rad protein assay measured at A_{595} (lower panel).
——, extract of the saline-treated rat stomach; – – – –, extract of the phenylbutazone-treated rat stomach.
Column size, 1 cm i.d. × 70 cm; solvent, 2% Triton X-100–50 mM Tris–HCl buffer (pH 7.4).

TABLE I. Effect of Fr. SM II on Each Peak Content in Extract of the Gastric Glandular Portion of Rats with Phenylbutazone- or Aspirin-Induced Ulceration

Experiments	Treatment	Dose (mg/kg)	Hexose content (μg/100 mg tissue wet wt.)		
			Peak I	Peak II	Peak III
(A) Intact rats	Intact[a]	—	582.2±63.3	294.4±39.9	410.8±65.3
	+Fr. SM II[b]	25	673.1±39.6	300.1±41.3	425.1±36.1
(B) Phenylbutazone (PB)-induced ulceration (5 h)	Intact[a]	—	582.2±63.3	294.4±39.9	410.8±65.3
	PB-control[a]	—	349.4±49.1[e]	280.3±40.9	393.6±44.3
	PB+Fr. SM II[c]	25	632.1±79.9[f]	288.6±47.3	430.2±73.3
(C) Aspirin (Asp)-induced ulceration (7 h)	Intact[a]	—	590.6±66.2	289.3±45.5	400.3±44.6
	Asp-control[a]	—	401.3±49.9[e]	285.3±50.1	377.3±39.8
	Asp+Fr. SM II[d]	25	599.1±66.3[g]	277.5±39.8	420.3±63.1

a) Saline. *b*) Sample was administered intraperitoneally 5.5 h before rats were sacrificed. *c*) Sample was administered intraperitoneally 30 min before the injection of phenylbutazone. *d*) Sample was administered intraperitoneally immediately after pylorus ligation. All values represent means±S.E. ($n=8$). Significantly different from each intact group: *e*) $p<0.05$. Significantly different from the PB-control group: *f*) $p<0.01$. Significantly different from the Asp-control group: *g*) $p<0.05$.

TABLE II. Chemical Composition of Each Peak

Peak I	Protein	21.8 (%)
	Carbohydrate	68.1
	Hexosamine	36.1
	Sialic acid	1.8
	Uronic acid	1.9
Peak II	Protein	56.6
	Carbohydrate	11.9
	Hexosamine	3.4
	Sialic acid	0.2
	Uronic acid	0.3
Peak III	Protein	52.2
	Carbohydrate	15.5
	Hexosamine	2.1
	Sialic acid	0.2
	Uronic acid	0.2

Hexosamine, sialic acid and uronic acid contents represent % of total weight.

affected by the drugs. Concomitant administration of Fr. SM II (25 mg/kg, *i.p.*) with phenylbutazone and aspirin brought the hexose content in peak I above that of the intact rats. The administration of Fr. SM II also increased the hexose content of peak I in intact rat stomach.

Chemical Composition of Glycoproteins Obtained from Gastric Glandular Portion of Intact Rat

Each fraction was chemically analyzed and the results are shown in Table II. Peak I was the most carbohydrate-rich fraction. The hexosamine content of peak I was no less than 50% of the total carbohydrates. The sialic and uronic acid contents of peak I were also higher than those of the other two peaks.

The relative ratio of these components remained the same while the total amount of carbohydrates was significantly changed by the administration of phenylbutazone, aspirin and Fr. SM II.

SDS- and disc-polyacrylamide gel (3.75%) electrophoretic analyses were performed. However, no peak was observed. This was probably due to the high molecular weight of peak I. On the other hand, a single glycoprotein peak was obtained (at pH 4.45) by the isoelectrofocusing electrophoresis of peak I.

Decrease of Peak I and Increase of Lipid Peroxide in Gastric Glandular Portion on Gastric Ulceration

Rats were sacrificed 3, 5 and 7 h after the administration of phenylbutazone (or 5, 7 and 9 h after the administration of aspirin). An increase of lipid peroxide was observed in both experimental ulcer models (Fig. 2). On the other hand, a decrease of peak I in the gastric glandular portion was noted (peaks II and III were not affected).

Effect of Fr. SM II on Lipid Peroxidation in Phenylbutazone- or Aspirin-Administered Rats

Lipid peroxide in the gastric glandular portion, serum and liver homogenate were measured. The amount of lipid peroxide in the gastric glandular portion was significantly increased in aspirin- or phenylbutazone-administered rats. The concomitant administration of Fr. SM II (25 mg/kg, *i.p.*) with phenylbutazone or aspirin lowered the lipid peroxide content significantly. The lipid peroxide in the serum was also decreased by the administration of Fr. SM II (Table III).

Effect of Glycoproteins on Lipid Peroxidation of Hepatic Microsomes, Arachidonic Acid Solution and Gastric Mucosa Homogenate

The effect of the glycoproteins on heat-induced lipid peroxidation was investigated using hepatic microsomes and arachidonic solution. As shown in Table IV, peak I had a potent lipid peroxidation inhibitory activity in both systems.

The effect of peak I on the lipid peroxidation of gastric mucosa homogenate was studied at various doses of peak I. As shown in Table V, lipid peroxidation was inhibited by peak I in a dose-dependent manner. The lipid peroxide level of peak I-treated gastric mucosa was

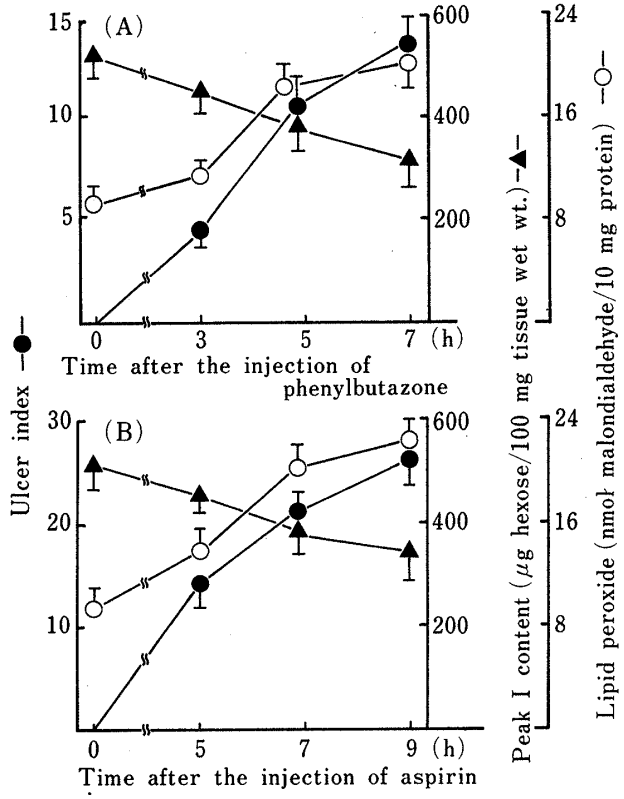

Fig. 2. Changes of Peak I and Lipid Peroxide Content in Gastric Glandular Portion Following the Induction of Gastric Ulcer

(A) phenylbutazone-treated rat stomach, (B) aspirin-treated rat stomach.

—●—, ulcer index; —▲—, peak I content (μg hexose/100 mg tissue wet wt.); —○—, lipid peroxide (nmol malondialdehyde/10 mg protein).

Each point represents the mean ± S.E. of five experiments.

Table III. Effect of Fr. SM II on Lipid Peroxidation in Gastric Glandular Portion, Liver and Serum of Rats with Phenylbutazone- or Aspirin-Induced Ulceration

Experiment	Treatment	Dose (mg/kg)	Gastric tissue (nmol malondialdehyde/10 mg protein)	Liver (nmol malondialdehyde/10 mg protein)	Serum (nmol malondialdehyde/10 mg protein)
(A) Intact rats	Intact[a]	—	10.1±1.5	3.9±0.6	1.9±0.3
	+Fr. SM II[b]	25	9.8±1.1	3.8±0.5	2.0±0.3
(B) Phenylbutazone (PB)-induced ulceration (5 h)	Intact[a]	—	10.6±1.4	4.0±0.8	2.0±0.5
	PB-control[a]	—	17.7±1.5[e]	4.2±0.4	3.2±0.6
	PB+Fr. SM II[c]	25	9.5±0.7[f]	4.1±0.8	2.6±0.9
(C) Aspirin (Asp)-induced ulceration (7 h)	Intact[a]	—	10.1±1.5	3.9±0.6	1.9±0.3
	Asp-control[a]	—	19.3±2.2[e]	4.3±0.4	3.0±0.5
	Asp+Fr. SM II[d]	25	10.3±1.1[g]	4.0±0.9	2.3±0.4

a) Saline. *b*) Sample was administered intraperitoneally 5.5 h before rats were sacrificed. *c*) Sample was administered intraperitoneally 30 min before the injection of phenylbutazone. *d*) Sample was administered intraperitoneally immediately after pylorus ligation. All values represent means±S.E. ($n=8$). Significantly different from each control group: *e*) $p<0.01$. Significantly different from the PB-control group: *f*) $p<0.001$. Significantly different from the Asp-control group: *g*) $p<0.01$.

Table IV. Effect of Each Peak on Lipid Peroxidation of Hepatic Microsomes and Arachidonic Acid Solution *in Vitro*

Treatment	Dose (μg/tube)	Malondialdehyde (nmol)	
		Microsomal fraction (/10 mg protein)	Arachidonic acid solution (/250 μg)
Control	—	6.57±0.39	6.01±0.48
Peak I	20	2.52±0.28[b]	2.21±0.32[b]
Peak II	20	4.99±0.51[a]	4.69±0.66
Peak III	20	5.33±0.50	4.92±0.38

The reaction mixture, consisting of a test fraction and 150 mM KCl–10 mM Tris–HCl buffer (pH 7.4), was incubated for 30 min at 37 °C. All values represent means±S.E. ($n=5$). Significantly different from the control group: *a*) $p<0.05$, *b*) $p<0.001$.

Table V. Effect of Peak I on Lipid Peroxidation of Hepatic Microsomes, Gastric Mucosa Homogenate and Arachidonic Acid Solution *in Vitro*

Treatment	Dose (μg/tube)	Malondialdehyde (nmol)		
		Microsomal fraction (/10 mg protein)	Gastric mucosa homogenate (/10 mg protein)	Arachidonic acid solution (/250 μg)
Control	—	7.53±0.31	1.99±0.11	6.01±0.53
Peak I	30	4.63±0.22[c]	0.85±0.10[c]	2.55±0.19[c]
	15	5.45±0.83	1.20±0.13[b]	3.91±0.45[a]
	5	6.60±0.51	1.39±0.09[b]	4.60±0.33

The reaction mixture, consisting of a test fraction and 150 mM KCl–10 mM Tris–HCl buffer (pH 7.4), was incubated for 30 min at 37 °C. All values represent means±S.E. ($n=8$). Significantly different from the control group: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

significantly lower than that of the control even at a dose of 5 μg/tube.

Stabilizing Effect of Peak I on Lysosomal Membrane

As inhibition of lipid peroxidation by peak I was demonstrated, the effect of peak I on the stability of the lysosomal membrane was next investigated. *N*-Acetyl-β-glucosaminidase

TABLE VI. Effect of Peak I on Release of *N*-Acetyl-β-glucosaminidase from Hepatic Lysosomes *in Vitro*

Treatment	*N*-Acetyl-β-glucosaminidase (nmol *p*-nitrophenol/mg protein/h)			
	Lysosomal fraction		Free enzyme	
	p-Nitrophenol	% inhibition	*p*-Nitrophenol	% inhibition
Control	3.75±0.44	—	4.22±0.23	—
Peak I (50 μg/tube)	1.99±0.32[a]	46.9	4.01±0.22	5.0
(20 μg/tube)	2.45±0.22	34.7	4.55±0.31	−7.8
0.01% Triton X-100	16.15±1.07[b]	−434.1	4.49±0.58	−6.4

All values represent means±S.E. ($n=5$). Significantly different from the control group: *a*) $p<0.05$, *b*) $p<0.001$.

was chosen to evaluate the stability of the lysosomal membrane. The enzyme activity detected in the supernatant after a 30 min incubation of the lysosome suspension was significantly lowered by the addition of peak I. It was confirmed that this effect was not caused by a direct interaction of peak I with the enzyme (Table VI).

Discussion

Since Hollander[17] claimed that mucus glycoproteins in the mucous layer were involved in the protection of gastric mucosa, many investigations have been carried out on this subject. HCO_3^- and mucus were reported to form a barrier against the assault of gastric acid.[18,19] In recent years, some glycoproteins have been suggested to be involved in the protection of the stomach from autodigestion; Azuumi *et al.*[5] reported the importance of glycoproteins in aspirin ulceration, and Takagaki and Hotta[20] reported that some glycoproteins showed an anti-peptic activity. In our previous paper, it was suggested that Fr. SM II might manifest its anti-ulcerogenic activity through an effect on glycoproteins.[2]

In the present work, in order to clarify the mechanism of the anti-ulcerogenic activity of Fr. SM II, the glycoproteins in the gastric glandular portion (which was affected by the administration of Fr. SM II) were fractionated on a Sepharose 6B column. It was demonstrated that the amount of peak I, which contained the highest molecular weight glycoproteins, was closely related to the status of ulceration (Table I and Fig. 2). Azuumi *et al.* also reported a relationship between high molecular weight glycoproteins (Fr. I) and ulceration. However, their glycoproteins were extracted from the whole stomach. Though we were not able to compare Fr. I and peak I directly, we assume that their Fr. I and our peak I contain overlapping glycoproteins. Histochemical observations of normal and ulcerated stomachs revealed that the amount of glycoproteins varied mainly on the gastric surface mucosal cells. Therefore, we concluded that it was preferable to study the change of the glycoproteins in the surface mucosal cells.

Since Takagaki and Hotta reported the existance of anti-peptic glycoproteins from rat stomach, the anti-peptic activity of peak I was measured. However, we could not detect any anti-peptic activity of peak I. Takagaki and Hotta claimed that their glycoproteins had a molecular weight of 95000, which means that they not be eluted in the flow-through fraction of the Sepharose 6B column. Therefore, it was assumed that peak I did not contain Takagaki's anti-peptic glycoproteins.

In the present paper, it was shown that peak I contains the major glycoproteins which were increased by the administration of Fr. SM II. Therefore, the relationship of the increase

of peak I and the anti-ulcerogenic activity was further investigated. Peak I was shown to have a lipid peroxidation inhibitory activity together with a lysosome-stabilizing activity. The role of lipid peroxidation in the induction of ulceration is not clear as yet. However, as shown in Fig. 2, the amount of lipid peroxide paralleled the ulcer index during the experiment. (A similar result was also observed when the reduced and carboxamide methylated human immunoglobulin G (IgG) was used as an anti-ulcer drug.[21]) It is natural to speculate that the increase of lipid peroxide level may be one of the causes of ulcer induction, and the lysosome-stabilizing activity (through the inhibition of lipid peroxidation) may therefore be involved in the mechanism of the anti-ulcer action.

It is desirable to administer an appropriate anti-ulcer drug depending on the cause of the ulceration. As shown in this article, the amount of peak I is related to the extent of the ulceration in the phenylbutazone- and aspirin-induced ulcer models. It might be possible to distinguish different types of ulceration and/or different types of anti-ulcer drugs in terms of the amount of peak I.

Acknowledgement We are grateful to Nippon Suisan Central Institute for providing ink bags of *Ommastrephes bartrami* LESUEL.

References

1) T. Mimura, K. Maeda, H. Hariyama, S. Aonuma, M. Satake and T. Fujita, *Chem. Pharm. Bull.*, **30**, 1381 (1982).
2) T. Mimura, K. Maeda, T. Terada, Y. Oda, K. Morishita and S. Aonuma, *Chem. Pharm. Bull.*, **33**, 2052 (1985).
3) H. Murakami, K. Oketani, H. Fujisaki, T. Wakabayashi, T. Ohgo and S. Okabe, *Jpn. J. Pharmacol.*, **32**, 299 (1982).
4) Y. Suzuki, M. Hayashi, M. Ito and I. Yamagami, *Jpn. J. Pharmacol.*, **26**, 471 (1976).
5) Y. Azuumi, S. Ohara, K. Ishihara, H. Okabe and K. Hotta, *Gut*, **21**, 533 (1980).
6) M. Dubois, K. A. Gills, J. K. Hamilton, P. A. Robers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
7) P. W. Holloway, *Anal. Biochem.*, **72**, 248 (1976).
8) J. Hiroi, T. Seki, M. Otsuka, S. Kastuki and F. Honda, *Jpn. J. Pharmacol.*, **31**, 144 (1981).
9) B. Gunner, *Acta Chem. Scand.*, **2**, 467 (1948).
10) L. Warren, *J. Biol. Chem.*, **234**, 1971 (1959).
11) T. Bitter and H. M. Muir, *Anal. Biochem.*, **4**, 330 (1962).
12) H. Ohkawa, N. Ohnishi and K. Yagi, *Anal. Biochem.*, **95**, 351 (1979).
13) K. Yagi, I. Nishigaki and H. Ohama, *Vitamins*, **37**, 105 (1968).
14) M. Sato, Y. Amikura, R. Aiimoto, R. Kimura and T. Murata, *Yakugaku Zasshi*, **98**, 757 (1978).
15) G. Weissman, *Biochem. Pharmacol.*, **14**, 525 (1965).
16) M. Ito, *Folia Pharmacol. Japon.*, **69**, 137 (1973).
17) F. Hollander, *Arch. Int. Med.*, **93**, 107 (1954).
18) S. E. Williams and L. A. Turnberg, *Gastroenterology*, **79**, 299 (1980).
19) A. Allen and A. Garnen, *Gut*, **21**, 249 (1980).
20) Y. M. Takagaki and K. Hotta, *Biochim. Biophys. Acta*, **584**, 288 (1979).
21) T. Mimura, M. Iwai, K. Maeda, I. Kohda, K. Tsujikawa and A. Aonuma, *J. Pharmacobio-Dyn.*, **7**, 718 (1984).