Proteolytic Activity of Human Gastric Mucosa.

I.

By

Mitsuhisa Hayakawa.

(早川 光久)

(From the Medical Clinic of Prof. T. Kurokawa, Faculty of Medicine, Tohoku University, Sendai.)

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In the precise study of the proteolysis of gastric mucosa (swine) Willstätter and Bamann¹⁾ found, besides pepsin (pH 2.0) proper, a proteinase named cathepsin by him which hydrolysed gelatin, clupein and egg albumin at pH 3.5–4.0. In addition to these he confirmed the ereptase activity (pH 8.0, leucyl-diglycin & diglycin splitting), but not tryptase activity, because the hydrolysis of gelatin at pH 8.0 was negative (48 hrs.).

Recently Hirooka²⁾ has in his studies with swine gastric mucosa, successfully separated the pepsin activity from the erepsin and cathepsin activity on one hand, and also the catheptic pepton splitting activity from erepsin by means of acid and alkaline treatment according to the method of Utzino and Sakai.³⁾ It will be interesting to study exactly the protease activity of the human gastric mucosa, by comparing with such results from experiments with animal materials.

EXPERIMENTAL.

Method and Materials.

1) Enzyme: Macroscopic normal gastric mucosa from cancer- and ulcer-stomach obtained by biopsy. Maceration 1+glycerin-water (1:1)3.

Acid and alkaline treatment: according to Utzino and Sakai.³⁾

Cysteine activation: 2 c.c. enzyme+0.024 grm. cystein-HCl in 1 c.c. H₂O (neutralized with NaOH), 37°C, 30 min., for control when needed, the volume of the original enzyme solution was made constant, with buffer solution.

2) Substrate: 1–2 % [or m/20-substrate-buffer-solution; acylpeptid or acylamino acid resolved at first in n/10 NaOH then added with buffer; buffer: m/10-citrate-n/10-HC or m/15-phosphate-buffer according to the conditions of experiments.

Reactions: 18–20 c.c. Substrate-buffer solution+2–3 c.c. enzyme

solution, under toluol, at 37°C; for control: without substrate or enzyme under the same condition; increased acidity in 4 c.c. digestate formol-titrated (0.1 n-NaOH). Only corrected values are given in the tables.

Results and Discussion.

I. *Hydrolysis of protein and pepton.*

Besides pepsin action (pH 2.0), hydrolyses of casein, serum albumin and egg albumin and also pepton between pH 4.0 and 5.0 are recognized (cathepsin action, Table I), which are with the addition of cystein apparently activated (pH 4.0 Table II). Further it must be attended that casein also at pH 8.5 is hydrolysed, while pepton without strangeness is (erepsin activity) (Table I).

TABLE I.
Proteolysis of Human Gastric Mucosa.
2 c.c. maceration juice; enzyme no. 173 & 179: cancer.

Increased acidity in 4 c.c. digestate, c.c. 0.1 n-NaOH										
Substrate. 18 c.c.	Time in hrs.	pH								
		2.0	3.0	4.0	4.4	4.6	5.0	6.0	7.5	8.5
1 % Casein (Hammarsten)	24	0.4	0.4	0.5	0.25	0.25	0.2	—	0.1	0.15
	48	0.5	0.4	0.55	0.35	0.35	0.3	—	0.2	0.2
	72	0.55	0.4	0.6	0.4	0.4	0.4	—	0.35	0.45
1 % Pepton (Kyokuto)	24	—0.05	—	0.35	—	0.2	—	0.3	0.6	0.3
	48	0.05	—	0.45	—	0.3	—	0.5	0.6	0.45
	72	0.1	—	0.5	—	0.55	—	0.6	0.9	0.55

After the acid treatment (pH 4.0, 40 min. 37°C) for the maceration disappears the erepsin activity, while pepton hydrolysing activity at pH 4.0 is reserved (Table III). And after the alkaline treatment (pH 7.7, 30 min., 37°C), the degrees of hydrolysis of casein, egg albumin and serum albumin at pH 4.0 become smaller than that of these substrates at pH 2.0–2.2, without being activated with cystein (Table V). On the contrary, the degrees of hydrolysis of these substrates at pH 4.0 by original maceration juice are similar to those at pH 2.0, and the former can be activated with cystein as has been seen in Table II (Table V), though the latter might not be. From these it is evident that the cathepsin action was removed by alkaline treatment. However, the pepton hydrolysing activity at pH 4.0 is reserved even after a stronger treatment with alkaline (pH 9.5, 2 hrs., 37°C (Table IV). This fact indicates that the hydrolyzing activity of casein and that of pepton at pH 4.0 differ from each other in

TABLE II.
Catheptase Activity of Human
Gastric Mucosa.
2 c.c. maceration juice; enzyme
no. 179: cancer; pH 4.0.

Increased acidity in 4 c.c. digestate, c.c. 0.1 n-NaOH			
Substrate 18 c.c.	Time in hrs.	With cystein	Without cystein
0.5 % Ovalbumin (Takeda)	24	0.2	0.1
	72	0.3	0.1
	120	0.3	0.15
1 % Ovalbumin (E. Merck)	24	0.4	0.3
	72	0.5	0.3
	120	0.6	0.4
1 % Serum albumin (E. Merck)	24	0.5	0.3
	72	0.5	0.4
	120	0.7	0.55
1 % Casein (Hammarsten)	24	0.65	0.4
	72	0.8	0.6
	120	0.9	0.65
1 % Pepton (Kyokuto)	24	0.4	0.1
	72	0.5	0.3
	120	0.65	0.35

TABLE III.
Catheptic Pepton Splitting Activity of
Human Gastric Mucosa
(free from ereptase activity).
3 c.c. maceration; enzyme: no. 173, cancer.

Increased acidity in 4 c.c. digestate; c.c. 0.1 n-NaOH						
Substrate 18 c.c.	Time in hrs.	pH				
		3.0	4.0	5.4	7.4	8.4
1 % Pepton (Kyokuto)	24	0.1	0.3	0.1	0	0
	48	0.15	0.4	0.25	0	0

TABLE IV.
Pepton Splitting Activity After
the Alkaline Treatment.
3 c.c. maceration; enzyme: no. 173, cancer.

Increased acidity in 4 c.c. digestate; c.c. 0.1 n-NaOH						
Substrate 18 c.c.	Time in hrs.	pH				
		3.0	4.0	5.4	6.8	7.8
1 % Pepton (Kyokuto)	24	0.05	0.15	0.05	0.1	0.2
	48	0.1	0.2	0.1	0.15	0.3
	72	0.1	0.3	0.15	0.15	0.35

relation to their resistency against alkaline. From these respects it may be concluded that in human gastric mucosa does exist catheptic pepton splitting enzyme (cathopeptonase) which was found by Sakai⁴⁾ for the first time in swine liver.

Furthermore, tryptase activity in gastric mucosa, which has been denied by Willstätter,¹⁾ probably exists in gastric mucosa, for the hydrolysis of casein at pH 8.5 is positive, which does not seem resulting from reversed and infiltrated pancreas trypsin, for the gastric mucosa employed was obtained from the cancer stomach affected with pyloric stenosis.

II. Hydrolyses of peptide and its derivatives.

The hydrolyses of diglycin and chloracetyl-l-leucin at pH 7.8 are clearly positive but that of chloroacetyl-l-phenylalanin is still smaller than those of the former (Table VI). Chloroacetyl-diglycin is hardly hydrolyzed by mucosa from cancer stomach, but a little by that from ulcer stomach

TABLE V.

The Pepsinase Activity of Human Gastric Mucosa
(free from the cathepsin activity).

2 c.c. maceration; enzyme: no. 173, cancer, alkaline treatment A (+), B (—).

Increased acidity in 4 c.c. digestate; c.c. 0.1 n-NaOH								
Substrate 18 c.c.	Enzyme	Time in hrs.	With cystein				Without cystein	
			pH				pH	
			2.2	3.0	4.0	5.0	0.4	2.0
1 % Casein (Hammarsten)	A	24	0.3	0.1	0.1	0.1	0.1	0.35
		48	0.4	0.2	0.1	0.15	0.15	0.4
		72	0.5	0.25	0.2	0.15	0.15	0.5
	B	24	0.4	—	0.65	—	—	0.4
		48	0.4	—	0.8	—	—	0.45
		72	0.6	—	0.8	—	—	0.5
1 % Ovalbumin (E. Merck)	A	24	0.3	—	0.2	—	0.2	0.3
		48	0.3	—	0.2	—	0.2	0.3
		72	0.6	—	0.3	—	0.25	0.4
	B	24	0.25	—	0.4	—	0.3	—
		48	0.3	—	0.4	—	0.3	—
		72	0.4	—	0.6	—	0.4	—
1 % Serum albumin (E. Merck)	A	24	0.45	—	0.25	—	0.2	—
		48	0.55	—	0.3	—	0.2	—
		72	0.6	—	—	—	0.3	—
	B	24	0.45	—	0.6	—	0.3	—
		48	0.55	—	0.6	—	0.45	—
		72	0.6	—	0.7	—	0.6	—

(Table VI). The extent of hydrolysis of these halogen-acyl-amino-acids by human gastric mucosa is nearly equal to that by intestinal juice (dog and also to that by intestinal mucosa (rabbit), which were observed in the experiments of Matsuo⁵⁾ and Hino⁶⁾ respectively. And it is of interest that it can be observed clearly even when the hydrolysis of casein at pH 8.5 is still yet questionable (24 hrs.) (Tables I & VI). From these results the existence of halogen-acylase activity in human gastric mucosa may be concluded. On the contrary the hydrolyses of benzoyl-diglycin and benzoyl-glycin were by no means recognized (Table VI).

Then influence of the kinase⁷⁾ activation was examined: hydrolysis of diglycin is not affected, that of benzoyl-diglycin is still questionable, but

TABLE VI.
Peptide Splitting Activity of Human Gastric Mucosa (1)
Experiments in Alkaline Side.
 2 c.c. maceration; pH 7.8.

Increased acidity in 4 c.c. digestate; c.c. 0.1 n-NaOH						
m/20 Substrate 18 c.c.	Time in hrs.	Enzyme, no.				
		196 cancer	194 cancer	177a cancer	207 ulcer	177b ulcer
Diglycin	24	1.60	1.70	1.50	1.60	1.40
	48	1.60	1.85	1.50	1.60	1.60
	72	1.70	1.95	1.85	1.65	1.60
Benzoyldiglycin	24	0	0.10	0	0	0
	72	0	0.10	0	0	0
	120	0.15	0.10	0.15	0.05	0
Chloracetyl-l-phenyl- alanin	24	0.30	—	—	0.50	—
	72	0.45	—	—	0.70	—
	120	0.60	—	—	0.80	—
Chloracetyl-l-leucin	24	0.60	0.45	0.65	1.00	0.95
	72	0.75	—	0.80	1.85	1.40
	120	1.20	—	0.85	2.10	1.70
Chloracetyldiglycin	24	0	—	0	0.25	0.25
	72	0.20	—	0	0.30	0.30
	120	0.20	—	0.25	0.40	0.35
Bezoylglycin	24	—0.05	—	0	0	0
	72	—0.10	—	0	0	0
	120	—0.15	—	0.10	0	—0.05

that of chlor-acetyl-diglycin seems to have undergone a little activation (Table VIII).

By the by, halogen-acylase activity of cancer stomach is weaker than that of ulcer stomach, while no difference with the dipeptidase activity is found between these two.

In the experiments in acid side, neither acid carboxypeptidase activity (benzoyl-diglycin, Waldschmidt-Leitz⁸⁾) nor acid acyl-dipeptidase activity (benzoyl-diglycin, Sakai⁹⁾) is found in human gastric mucosa. This is also the case still with cystein activation (Table VII).

SUMMARY.

1. In human gastric mucosa besides peptic action, are found cathepsin action (pH 4.0–5.0, casein, egg albumin and serum albumin) and catheptic

TABLE VII.

Peptide Splitting Activity of Human Gastric Mucosa (2)
Experiments in Acid Side.

3 c.c. maceration juice; cystein activation A (+), B (—); pH 4.2–4.6.

Increased acidity in 4 c.c. digestate; c.c., 0.1 n-NaOH								
m/20 Substrate 18 c.c.	Time in hrs.	Enzyme, no, (pH)						
		no. 225 ulcer (4.4)		no. 233 ulcer (4.2)	no. 209 cancer (4.6)		no. 196 cancer (4.5)	no. 194 cancer (4.5)
		A	B	B	A	B	A	A
Diglycin	24	0.10	0	0	—	—	—0.05	0
	72	0.10	0	0	—	—	—0.10	0
	120	—	—	—	—	—	—0.10	0
Chloracetyl-l- phenylalanin	24	0.05	—	—	0.05	0	0	—
	72	0.10	—	—	0.05	0.05	—0.05	—
	120	—	—	—	—0.10	0.05	—0.10	—
Chloracetyldiglycin	24	0.05	0	—	—	0.05	0	—
	72	0.05	0	—	—	0.05	0.05	—
	120	—	—	—	—	0.05	—0.05	—
Chloracetyl-l-leucin	24	0	0	0	0	0	—0.05	—
	72	0.05	0	0	0	0	—0.05	—
	120	—	—	—	—0.05	0.05	—0.10	—
Benzoyldiglycin	24	0.05	0	—	0.05	0.10	0	0
	72	0.05	0	—	0.05	0.10	0	0
	120	—	—	—	—0.10	0.20	—0.10	—0.05
Benzoylglycin	24	0.05	0	—	—	—	0	—
	72	0.05	0.05	—	—	—	0	—
	120	—	—	—	—	—	—0.05	—

pepton splitting action (cathopeptonase). And casein is also at pH 8.5 hydrolyzed (72 hrs.).

2. I have succeeded also with human gastric mucosa to separate the pepsin action from the cathepsin action, and also the cathopeptonase action from the erepsin action by means of acid and alkaline treatment respectively according to the methods of Utzino and Sakai.³⁾

3. In human gastric mucosa halogen-acylase action (pH 7.8, chlor-acetyl-l-leucin and chloracetyl-l-phenylalanin) is found, but carboxypeptidase action (pH 7.8, benzoyl-diglycin, with kinase) or hippuricase action (pH 7.8, benzoyl-glycin) not. The hydrolysis of chlor-acetyl-

TABLE VIII.

Peptide Splitting with Kinase Activation.

2 c.c. maceration + 0.05 grm. kinase pulver, 37°C 30 min.; enzyme:

No. 204, ulcer; m/20-substrate; Increased acidity in 4 c.c.

digestate; c.c., 0.1 n-NaOH; pH 7.8.

Substrate 18 c.c.	Time in hrs.	Without activation	With activation
Diglycin	24	1.30	1.50
	48	1.40	1.75
	72	1.45	—
Benzoylglycin	24	0	0.20
	48	0	0.20
	72	—0.05	—
Chloracetyldiglycin	24	0.10	0.50
	48	0.30	0.60
	72	0.40	—

diglycin at pH 7.8 by gastric mucosa from ulcer stomach is certain extent recognized.

4. Neither acid carboxy-peptidase action nor acid acyldipeptidase action (pH 4.2–4.6, benzoyl-diglycin, chloracetyl-diglycin) despite of the cystein activation is still yet found. The hydrolysis of diglycin, chloracetyl-l-leucin, chloracetyl-phenylalnin and benzoyl-glycin at pH 4.5 with cystein, all of these are negative.

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