Radioimmunoassay of pepsinogens I and II in human serum*

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Abstract Radioimmunoassay (RIA) for pepsinogens I and II (PG I and II) in serum is developed by using the purified PG I and II from human gastric mucosa. The assay range was 8~256μg/L for PG I and 2~64μg /L for PG II, the sensitivity was 1.3 and 0.6μg/L, respectively. The reproducibilities of PG I -RIA (106.9%) and PG II-RIA (106.7%) are quite well. The within- and between-assay coefficient of variation (CV) of each RIA was 5.1% and 6.3% for PG I, 7.2% and 8.9% for PG II. The serum PG I level in healthy volunteer was 60.41±14.98μg/L (mean±SD), significantly higher than the serum PG II level which was 20.70±9.64μg/L. Therefore, the PG I-RIA and PG II-RIA are useful tools in studying the relation between serum pepsinogen levels and various peptic disorders.

Keywords Pepsinogen, Radioimmunoassay, Stomach cancer, Tumor marker, ^{125}I

1 Introduction

Pepsinogens produced by human gastric mucosa are classified into two immunologically distinct groups[1,2], pepsinogen I (PG I), which is derived from chief and mucus neck cells, and pepsinogen II (PG II), which is derived from cardiac glands, pyloric glands and Brunner's glands as well as chief and mucus neck cells[3,4]. The two pepsinogens not only are secreted into the lumen of the gastrointestinal tract, but also enter the blood circulation. It is believed that the blood pepsinogen level reflects the morphological and functional status of the gastric mucosa. Many studies show that changes in human serum pepsinogen level are associated with many gastric diseases, especially gastric-duodenal ulcer and gastric cancer.[5,6] It has been reported recently that the serum pepsinogen level was valuable in gastric cancer diagnosis.[7] The assay method they used was to measure total proteolytic activity of serum at acid pH. In this paper, PG I-RIA and PG II-RIA using purified PG I and II from surgically resected stomaches are described, and Chinese serum concentrations of PG I and PG II in normal controls are presented for the first time.

2 Materials and methods

2.1 Purification of PG I and II from human gastric mucosa

The surgically resected stomaches were free from the invaded part, and the mucosa was separated by sharp dissection. After the mucosa was homogenized in the phosphate buffer, the homogenate was centrifuged at 18 kg and at 4°C for 30 min. The pepsinogens in supernatant were purified using DEAE-52 chromatography and gel filtration HPLC (Bio-Sil SEC 125, Bio-Rad). The PG I and II were separated from each other by Q2 anion exchange chromatography (Bio-Rad) in the last step. The purity and the molecular mass of PG I and II were determined by SDS-PAGE and the potential proteolytic activity was measured using bovine hemoglobin as substrate.[8]

2.2 Preparation of the antiserum to purified PG I and PG II

Antiserum to PG I and PG II was raised in New Zealand white rabbits by subcutaneous injecting. The antigens containing the purified PG I (or PG II) of 1 mg was diluted in PBS (pH7.4) and emulsified with Freund's complete adjuvant of an equal volume. After one month, booster injections of 300~500μg antigen in Freund's incomplete adjuvant were administered every two weeks for 3 months. The dilution of the antiserum was checked with double immunodiffusion and the affinity constant was

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determined by Scatchard scaling.

2.3 Radioiodination of the PG I and PG II

The reaction mixture contained 5μg PG I (or PG II), 20μl phosphate buffer (0.2 mol/L, pH7.6), 3.7 MBq of $Na^{125}I$ and 20μg(20μl) chloramine-T. The 100μg (50μl) sodium metabisulfite was added to terminate the reaction after 1 min. The free ^{125}I was separated from ^{125}I-PG I (or ^{125}I-PG II) with a sephadex G-50 column which was saturated with 1% bovine albumin solution in elution buffer before use.

2.4 Radioimmunoassay procedure for PG I and PG II

The diluent buffer was 50 mmol/L phosphate buffer, pH7.3, containing 0.5% bovine albumin. The PG I standards, antiserum and ^{125}I-PG I were all diluted with this buffer. The initial incubation mixture in reaction tubes contained 100μl diluent buffer, 100μl PG I standard or test serum, 100μl anti-PG I (in 1:1000 final dilution) and 100μl ^{125}I-PG I (approximately 20000 cpm). Then the mixture was kept at 4°C for 24 h, the goat anti-rabbit gamma globulin was added in excess of that required to precipitate all of the rabbit gamma globulin and the mixture was incubated at 37°C for 30 min before the tubes were centrifuged. The supernate was decanted and the radioactivity in the precipitate was counted. All standards and samples were tested in duplicate.

The RIA procedure for PG II was the same as the above, except the anti-PG II in a 1:15000 final dilution.

2.5 Serum samples

Serum samples were obtained from subjects free from abdominal complaints and without evidences of gastroduodenal disorder, liver disease and renal disease after health examination.

3 Results

3.1 Purification of PG I and PG II

Purification results are shown in Table 1. The molecular mass of PG I and II are 42000 and 37000, respectively. Both of two pepsinogens show the highest proteolytic activity at pH1.8. PG II is much more resistant to alkali than PG I, which is inactivated at pH value higher than 8. PG I and PG II can be activated to pepsins at acid pH. These biochemical characterizations of PG I and PG II are similar to other reported results.[8,9]

Table 1 Purification of PG I and PG II from human gastric mucosa

Procedure	Total protein /mg	Total activity /U	Specific activity /U·mg^{-1}	Yield /%
Crude extract	130.3	418.8	3.2	100
DEAE-52	34.9	366.5	10.5	87.5
HPLC	18.6	266.0	14.3	63.6
Q-2: PG I	10.5	131.3	12.5	31.3
PG II	2.2	42.0	19.1	10.0

3.2 The antiserum to PG I and PG II

The final dilution of the antiserum we prepared is 1:1000 for anti-PG I and 1:15000 for anti-PG II. The affinity constant K_a is 4.82×10^9L/mol for anti-PG I and 5.78×10^9L/mol for anti-PG II. The cross reaction between them is less than 5%. The antiserum also does not react with other enzymes (i.e. trypsin and cathepsin) or antigens used in some RIAs (i.e. AFP, CEA and AIF).

3.3 The standard curves for RIA of PG I and PG II

Fig.1 shows the standard concentration curves of PG I and PG II in the ranges of 8~256 μg/L for PG I -RIA, 2~64μg/L for PG II-RIA.

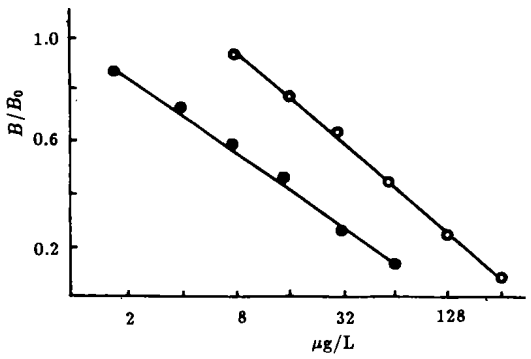

Fig.1 Standard curves of PG I-RIA and PG II-RIA
o—o PG I; •—• PG II

3.4 Characterization of PG I and PG II RIAs

The sensitivity, corresponding to 2×SD less than the mean counts bound in 10 zero standard tubes, was 1.3μg/L for PG I-RIA and

0.6μg/L for PG II-RIA. Sera were assayed before and after addition of known amounts of standards. Three different standards (low, medium, high) were added. The average recoveries were 106.9% for PG I and 106.7% for PG II. The mean within-assay CV was 5.2% for PG I and 7.2% for PG II by analyzing the eight same tubes at the same time. The between-assay CV was 6.1% for PG I and 8.9% for PG II by analyzing the same serum for three times. After being storaged at 4°C for one month, the zero standard binding of ^{125}I labeled PG I and PG II were more than 40% of the total radioactivity.

3.5 Serum PG I and PG II levels in control subjects

The mean serum PG I level was 60.4±14.48μg/L (mean±SD) in 60 control subjects. The mean serum PG II level was 20.70±9.64μg/L (mean±SD).

4 Discussion

The final dilution of anti-PG I is 1:1000, much lower than that of anti-PG II (1:15000) after eight times of booster immunization. The same phenomenon exists in other studies of pepsinogens. The explanation of this weaker antigenicity may possibly be that the molecular structure of PG I does not give rise to strong immunological reaction for rabbits. Both of the PG I and PG II levels in human serum are in the ranges of their standards and the serum samples could be assayed without dilution. The cross reaction between PG I and PG II can be neglected. Thus, the assay systems we established here are sensitive and specific for analysing PG I and PG II levels in human serum and are satisfactory for clinical use.

The data of this study show that PG I concentration in control human serum is significantly higher than that of PG II. The amount of PG I antigen we purified from gastric mucosa is about three times greater than PG II. It suggests that the higher concentration of PG I is, at least in part, due to the higher rate of synthesis and release of PG I into blood circulation compared with PG II. The concentrations of PG I and PG II in contol subjects by our assay are similar to those reported by Samloff[10] (PG I, mean=62.9μg/L; PG II, mean=10.8μg/L) and higher than those reported by Ichinose *et al*[5] (PG I, mean=43.6μg/L; PG II, mean=15.3μg/L), though these data can not be compared without calibration of the standards used by comparable methods in each RIA system. More serum samples of control subjects should be tested by using our RIA method.

Although the result reported by Cheng Zhao-Ming *et al.*[7] indicated that the serum pepsinogen levels of gastric cancer patients were much lower than those of control subjects, they used proteolytic assay method to detect both PG I and PG II, as well as other proteases that may be present in serum. So the RIA method based on the specific antigen-antibody reaction must be used in such assays. It needs further studies whether the serum pepsinogen level can be used as a tumor marker[6,11] of gastric cancer.

References

1 Kusher I, Papp W, Burlin P . J Clin Invest, 1964, 43:1983
2 Samloff IM. Gastroenterology, 1971, 60:586
3 Samloff IM. Gastroenterology, 1971, 61:185
4 Samloff IM, Liebman WM. Gastroenterology, 1973, 65:36
5 Ichinose M, Miki K, Furihata C *et al.* Clinica Chimica Acta, 1982, 126:183
6 Stenmernam GN, Samloff IM, Nomure AMY *et al.* Clinica Chimica Acta. 1987, 163:191
7 Cheng Z M, Miao C L, Zhang Y C *et al.* J of Zhenjiang Medical College, 1996, 16:77
8 Jiang M J, Xiao Z J, Huang X Q *et al.* Chinese J of Experiment & Clinical Immunology, to be published
9 Furihata C, Saito D, Fujiki H *et al.* Eur J Biochem, 1980, 105:43
10 Samloff IM. Gastroenterology, 1982, 82:26
11 Yamaguchi T, Takahashi T. Yokuta T *et al.* Cancer, 1991, 68:906