The cytotoxic and ACE-inhibitory activities of promod 278P hydrolysate of ovotransferrin

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Summary and Implications

The objective of this study was to investigate the cytotoxic and ACE-inhibitory activities of ovotransferrin and its promod 278P enzyme hydrolysate. Ovotransferrin from egg white was hydrolyzed using promod 278P at 45 °C for 3 h. The cytotoxicity and ACE-inhibitory activity was determined. The promod 278P hydrolysate of ovotransferrin showed a potent cytotoxicity (> 90%) at 20 mg/mL in all cancer cell lines tested. The IC_{50} value of the promod 278P hydrolysate of ovotransferrin was 1.53 ± 0.20 mg/mL. The promod 278P hydrolysate of ovotransferrin showed a strong ACE-inhibiting activity, but ovotransferrin did not. This result indicated that the promod 278P hydrolysate of ovotransferrin have a great potential as an anticancer and antihypertension agent for humans, but the information on the peptides responsible for the functional activities is not available yet.

Introduction

Peptides derived from ovotransferrin were reported to have antimicrobial, antihypertensive, antioxidant, and anticancer activities. The antibacterial peptide mixture purified from ovotransferrin by pepsin digest had a strong antibacterial activity and the minimum inhibitory concentration against *Staphylococcus aureus* and *Escherichea coli* was 0.06 and 0.1 mg/mL, respectively. The autocleaved ovotransferrin had strong antitumor activities toward colon and breast cancer cells. The enzyme hydrolysates of ovotransferrin showed protective effects against the oxidative stress-induced DNA damage in human leukocytes. Also, peptides derived from ovotransferrin using thermolysin with sonication showed a strong ACE-inhibitory activity. The mass spectrometric analysis of the hydrolysate indicated that 99% of the ovotansferrin peptides had mass sizes smaller than 6 kDa and only 1% was larger than 10 kDa. The hydrolysis of egg white using thermolysin produced bioactive peptides with high ACE-inhibitory and antioxidant activities. The enzyme suitable for hydrolysis may in principle be any food-grade proteases or protease mixtures from a variety of sources such as plants, animals, or microorganisms like a fungi or bacteria. Suitable proteases are commercially available. Among the food-grade enzymes, Promod 278P comprises proteinase from Carica papaya (papain) and proteinases and peptidases from *Bacillus subtilis*. Promod 278P was particularly effective with respect to extracting the protein from the milled maize. The objective of this study was to test the ACE-inhibitory and cytotoxic activities of promod 278P hydrolysate of ovotransferrin.

Materials and Methods

Lyophilized ovotransferrin (pH 6.5) was dissolved in distilled water at 20 mg/mL concentration and hydrolyzed at 45 °C for 3 h using promod 278P (E.C. 3.4.24.28, endopeptidase). At the end of hydrolysis, the enzyme was heat inactivated at 100 °C for 10 min, and then centrifuged. The resulting supernatant was freeze-dried, and the powder was used as the promod 278P hydrolysate of ovotransferrrin (MTT assay). The size of the promod 278P hydrolysate of ovotransferrrin was determined using SDS. ACE inhibitory activity was determined using hippury-hisidyl-leucine (HHL) and angiotensin-converting enzyme (ACE). Human normal and cancer cell lines, including MCF-7 (human breast adenocarcinoma), HT-29 (human colon adenocarcinoma), LoVo (human colon adenocarcinoma), HeLa (human cervix adenocarcinoma) and HepG2 (human liver hepatoblastoma), were used to test the *in vitro* cytotoxicity of the ovotransferrin hydrolysates.

Statistical Analysis: All results were presented as mean ± SE, and statistical analysis was performed using the SPSS package for Windows (version 18.0). The IC_{50} value was calculated using Soft Max Pro (version 6.3). The mean values were compared using the one-way ANOVA followed by Duncan's multiple range tests ($P < 0.05$).

Results and Discussion

Hydrolysis of Ovotransferrin: Figure 1 shows the SDS-PAGE profiles of natural ovotransferrin and the ovotransferrin hydrolysate using promod 278P. The natural ovotransferrin showed several bands as such: one heavy band near 75 kDa, and the other light bands near 50 and 37 kDa positions. The purity of natural ovotransferrin used was > 85%. The SDS-PAGE of promod 278P enzyme hydrolysate resulted in one clear band at the bottom of the gel (< 10 kDa). The promod 278P enzyme hydrolyzed ovotransferrin well and produced peptide with molecular weight < 10 kDa (Figure 1).

ACE-Inhibitory Activity: The promod 278P hydrolysate of ovotransferrin had stronger ACE-inhibitory activity than

the natural ovotransferrin. Increasing the concentration of ovotransferrin from 5 to 20 mg/mL did not increase the ACE-inhibitory activity of ovotransferrin. However, the promod 278P hydrolysate of ovotransferrin showed 76.82 ± 1.28% ACE-inhibitory activity at 10 mg/mL level, and that at 5, 2.5, 1.25, 0.625, and 0.3125 mg/mL level showed 73.33 ± 2.56%, 56.85 ± 1.84%, 50.32 ± 3.71%, 17.30 ± 0.13%, and 4.52 ± 6.83% ACE-inhibitory activity, respectively. The IC_{50} value of promod 278P hydrolysate of ovotransferrin was 1.53 ± 0.20 mg/mL (Figure 2). Many ACE-inhibitory peptides have been found in various egg white proteins, including ovotransferrinand ovalbumin.

In Vitro Cytotoxicity of Ovotransferrin and Promod 278P Hydrolysate of Ovotransferrin: The MTT assay results of ovotransferrin and the promod 278P hydrolysate of ovotransferrin on various cancer cell lines are shown in Figure 3. The results expressed as IC_{50} means the effective concentration of test samples required for 50% cytotoxic activity under the experimental conditions (Table 1). Most anticancer agents do not differentiate between cancerous and normal cells, leading to systemic toxicity and adverse effects. Therefore, we decided to test both ovotransferrin and the promod 278P hydrolysate of ovotransferrin against normal and cancer cell lines. The cytotoxic effect of ovotransferrin and the promod 278P hydrolysate of ovotransferrin on normal cell (MRC-5) was significantly different from that on human cancer cell lines. At all concentrations tested, both ovotransferrin and promod 278P hydrolysate of ovotransferrin showed < 20% of cytotoxic activity against MRC-5 cells (data not shown), indicating that both had no cytotoxicity to the normal MRC-5 cells. Figure 3 indicated that increasing the concentration of ovotransferrin from 5 to 20 mg/mL did not increase its cytotoxic activity against all cancer cell lines. However, the enzymatic hydrolysate of ovotransferrin showed significant cytotoxic effects against all cancer cell lines tested. Cytotoxic effects of ovotransferrin and the promod 278P hydrolysate of ovotransferrin to HeLa cells are shown in Figure 3(B). The promod 278P hydrolysate of ovotransferrin inhibited the growth of HeLa cells by 80, 94, and 95% at concentrations 5, 10, and 20 mg/mL, respectively (Figure 3B). The IC_{50} value of the promod 278P hydrolysate of ovotransferrin on HeLa cell was 3.45 mg/mL. Cytotoxic activity was only weakly detected on HepG2 cancer cell after being treated with ovotransferrin (Table 1). However, the promod 278P hydrolysate of ovotransferrin at 5 mg/mL showed more than 50% cytotoxic activity (54.51%) on HepG2 cancer cell. The IC_{50} values of ovotransferrin and the promod 278P hydrolysate of ovotransferrin on HepG2 cells were over 40 mg/mL and 4.43 mg/mL, respectively. Among the cancer cell lines, MCF-7 and LoVo cells were the least sensitive to ovotransferrin and the promod 278P hydrolysate of ovotransferrin (Figure 3A and 3E). Ovotransferrin also has antioxidant, antimicrobial and cytotoxic activities, but the enzyme hydrolysates of ovotransferrin showed stronger cytotoxic effects than natural ovotransferrin against human cancer cell lines of various tissue origins, including the lung (A549 and SK-MES-1), stomach (AGS), breast (MCF-7), larynx (Hep-2), cervix (HeLa), and liver (HepG2).

Conclusion

The promod 278P hydrolysate of ovotransferrin has stronger ACE-inhibitory and cytotoxic activities than ovotransferrin. These results suggested that ovotransferrin-promod 278P hydrolysate can be used as a good natural functional substance for humans.

Table 1. The concentrations inhibiting 50% of the cell growth (IC_{50}) value of ovotransferrin and the promod 278P hydrolysate of ovotransferrin on various human cancer cell lines[1].

Sample \ Cancer cells	IC_{50}[2] (mg/mL)				
	MCF-7	HeLa	HepG2	HT-29	LoVo
Ovotransferrin	26.96±1.61	32.79±8.84	>40	30.89±2.18	28.96±1.51
Promod 278P hydrolysate	10.05±1.55	3.45±0.94	2.43±1.87	4.92±0.63	10.34±3.91

[1]Values are means with SE (n = 3).

[2]IC_{50} value means the effective concentration of phosvitin required for 50% cytotoxic activity under the experimental condition. MCF-7, human breast adenocarcinoma; HeLa, human cervix; HepG2, human liver hepatoblastoma; HT-29, human colon adenocarcinoma, LoVo, human colon adenocarcinoma.

Figure 1. SDS-PAGE band pattern of ovotransferrin and the promod 278P hydrolysate of ovotransferrin. Lane 1: marker; lane 2: natural ovotransferrin; lane 3: promod 278P hydrolysate of ovotransferrin.

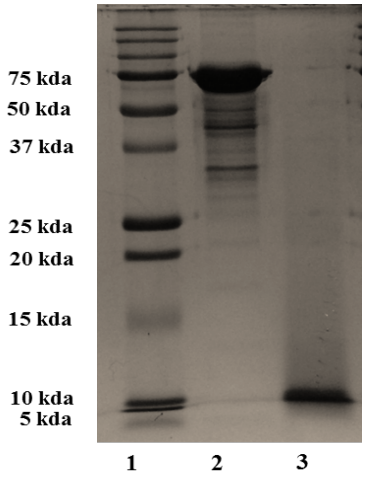

Figure 2. The angiotensin-converting enzyme (ACE) inhibitory effect (%)[1] of ovotransferrin (A) and the promod 278P hydrolysate of ovotransferrin (B). [1]Values not sharing the same letter are significantly different from one another (P<0.05).

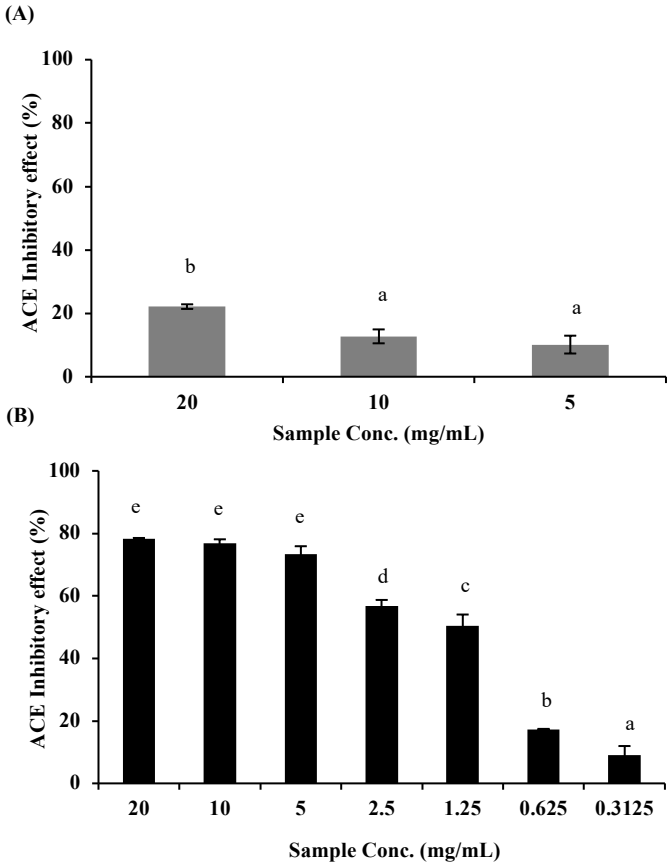

Figure 3. The cytotoxic effects of ovotransferrin (▩) and the promod 278P hydrolysate of ovotransferrin (■) on various human cancer cell lines by 3-[4,5-dimethythiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. (A) Human breast adenocarcinoma (MCF-7) cell, (B) Human cervix adenocarcinoma (HeLa) cell, (C) Human liver hepatoblastoma (HepG2) cell, (D) Human colon adenocarcinoma (HT-29) cell, and (E) Human colon adenocarcinoma (LoVo) cell. Values are mean with standard error.