Antioxidant, Antimicrobial, and Cytotoxic Activities of Ovotransferrin from Egg White

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Abstract

The antioxidant, antimicrobial, and cytotoxic activities of ovotransferrin were investigated in vitro. The antioxidant capacity of ovotransferrin was evaluated using the 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging method, antimicrobial effects using the agar well diffusion method, and cytotoxicity using the 3-(4,5-dimethylthizol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The DPPH radical-scavenging capacity of ovotransferrin at 1 mg/mL level reached approximately 60% after 48 h of reaction. The antimicrobial effects of ovotransferrin against common food-borne pathogens, Staphylococcus aureus KCCM 32395, Bacillus cereus KCCM 40935, Listeria monocytogenes ATCC 15313, Escherichia coli O157:H7 ATCC 43895, and Helicobacter pylori HpKCTC 26695 were dose dependant. Gram-positive bacteria was more sensitive to ovotransferrin than gram-negative bacteria. Ovotransferrin showed stronger antimicrobial effect against L. monocytogenes than other gram-positive bacteria tested. The cytotoxicity of ovotransferrin was evaluated in human cancer cell lines, various tissue origins, including the larynx (Hep-2), stomach (AGS), lung (SK-MES-1), liver (HepG2), breast (MCF-7), cervix (HeLa), and colon (HT-29). Ovotransferrin displayed relatively high cytotoxicity (≤60% inhibition effects) at 40 mg/mL. At lower concentrations (≤10 mg/mL), however, ovotransferrin cytotoxic effects were not significant in all cancer cell lines tested. These results indicated that ovotransferrin has potential to be used as an antioxidant or antimicrobial agent in foods or a pharmaceutical agent against cancers.

Key words: ovotransferrin, antioxidant activity, antimicrobial activity, cytotoxic activity

Introduction

Chicken egg has been used as an important source for protein, energy, vitamins, and minerals for human (Yi et al., 2003), and is an excellent source for biologically active substances (Mine, 2007). Ovotransferrin from egg white was first characterized by Schade and Caroline (1944) who called it conalbumin, subsequently renamed as ovotransferrin after being confirmed as an iron-binding protein, and became a member of the transferrin family (Williams, 1968; Yamamoto, 1997).

Ovotransferrin accounts for approximately 12% of egg white protein and is best known for its ability to bind iron. It is comprised of 686 amino acids with 77.9 kDa molecular weight and an isoelectric point of 6.38. It contains 15 disulfide cross-links and has no free sulfhydryl groups (Wu et al., 2011). Ovotransferrin is reported to have antimicrobial, antioxidative, antiviral, and immunomodulatory activities (Giansanti et al., 2005; Rath et al., 2009; Schade and Caroline, 1944; Valenti et al., 1985; Xie et al., 2002). The antimicrobial activity of ovotransferrin was recognized soon after it was first purified (Schade and Caroline, 1944). Since then, the antibacterial properties of ovotransferrin against a variety of pathogens including Escherichia coli, Pseudomonas spp., Strep- tococcus mutans, Staphylococcus aureus, Bacillus cereus, Salmonella Enteridis, and Candida spp. have been reported (Ko et al., 2008a; Ko et al., 2008b). The iron-binding capacity of ovotransferrin was initially believed to be responsible for its antibacterial activity, but antimicrobial mechanisms of ovotransferrin are not fully defined yet (Ibrahim, 1997; Ibrahim et al., 2000).

It has previously been reported that the antioxidant activ-
ity of ovotransferrin exhibits superoxide dismutase-like activity, which is promoted by its metal binding activity (Ibrahim et al., 2007; Shen et al., 2010), but the presence of free radical scavenging activity in ovotransferrin has not been reported. Ibrahim and Kiyono (2009) demonstrated that ovotransferrin autocleaved under reducing conditions (reduction with 0.2 mM tris (2-carboxyethyl) phosphine in 20 mM citrate-phosphate, pH 4.0, for 6 h at 37°C) exhibited stronger SOD-like activity, and anticancer effects against human colon and breast cancer cells than the natural ovotransferrin.

The objective of this study was to determine the antioxidant and antimicrobial capacities of ovotransferrin, and its cytotoxic effects against human cancer cells.

Materials and Methods

Materials

The apo-ovotransferrin (iron-free) used in this study was separated from chicken eggs using the method by Ko and Ahn (2008). 2,2-Diphenyl-1-picryl hydrazyl (DPPH), L-Ascorbic acid, and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide were purchased from Sigma Chemical Co. (St. Louis, USA), RPMI 1640 medium, Dulbecco’s modified Eagle’s medium (DMEM) medium, minimum essential medium, fetal bovine serum (FBS), horse serum, and penicillin-streptomycin were from Gibco-BRL (Grand Island, USA) and tryptic soy broth (TSB), bacto peptone, yeast extract, and brucella agar were from Difco Laboratories (Sparks, USA).

DPPH radical-scavenging activity

The DPPH radical-scavenging activity was determined by the method of Xu et al. (2007), with some modifications. Briefly, 1 mL of 0.5 mM DPPH ethanol solution was mixed with 1.5 mL of ethanol and added to 2.5 mL of sample solution at different concentrations. This mixture was maintained at 37°C during 72 h, and absorbance at 517 nm was measured at every 12 h. L-Ascorbic acid (0.5 mg/mL) was used as a reference compound. The control was the absorbance of 2.5 mL of sample solution mixed with 2.5 mL of ethanol, and the blank was the value for 2.5 mL of water mixed with 1.5 mL of ethanol and 1 mL of 0.5 mM DPPH ethanol solution. The residual radicals were calculated as follows:

Residual radicals (%) \[= 1 - \left( \frac{\text{Absorbance of sample} - \text{Absorbance of control}}{\text{Absorbance of the blank}} \right) \times 100 \]

Bacterial strains and culture conditions

Bacterial strains Staphylococcus aureus KCCM 32395, Bacillus cereus KCCM 40935, Listeria monocytogenes ATCC 15313, and Escherichia coli O157:H7 ATCC 43895 were purchased from the Korean Culture Center of Microorganisms (KCCM, Korea). Helicobacter pylori HpKCTC 26695 was obtained from the H. pylori Korean Type Culture Collection (HpKCTC) (Jinju, Korea). Each strain was cultured through 2 consecutive 24 h growth cycles in tryptic soy broth supplemented with 0.6% yeast extract (TSB-YE) at 37°C and used for further studies. H. pylori was activated in brucella agar plates supplemented with 5% (v/v) horse serum and was cultured under micro-aerophilic conditions (10% CO₂ atmosphere) for 3 d. For these studies, the strains were then inoculated in brucella broth supplemented with 5% horse serum and cultured for 1 d at 37°C before use.

Agar well diffusion assay

Inoculum of each strain was prepared in TSB-YE and incubated overnight at 37°C. All bacteria were suspended in 0.1% sterile peptone water and diluted to 10² CFU/mL. One milliliter of each bacterial suspension was added to 99 mL of 0.75% soft TSB-YE agar or brucella agar, after which 20 mL of inoculated agar was poured into a petridish. Once the agar plates solidified, 6-mm diameter wells were aseptically cut with a sterilized cork borer. The freeze-dried ovotransferrin samples were dissolved in distilled water to a final concentration of 80 mg/mL. All solutions prepared were sterilized using 0.22 µm syringe filters before performing this experiment. Hundred-microliter aliquots of the ovotransferrin solutions were subsequently delivered onto the dried TSB-YE agar or brucella agar plates. The TSB-YE agar plates were then incubated at 37°C for 24 h, and brucella agar plates were cultured under micro-aerophilic conditions (10% CO₂ atmosphere) for 2-3 d. The antimicrobial activity of ovotransferrin against each bacterial strain was estimated via the observation of clear zones (Taye et al., 2011; Voidsarou et al., 2011). Three replicates were prepared per sample.

Cell lines and culture conditions

Human cancer cell lines were purchased from the Korean Cell Line Bank (KCLB; Seoul National University, Seoul, Korea). The HT-29 (human colon adenocarcinoma), AGS (human stomach adenocarcinoma), and MCF-7 (human breast adenocarcinoma) cell lines were maintained in RPMI 1640 (Gibco Laboratories, Grand Island, USA) containing 10% heat-inactivated FBS (HyClone, USA), pen-