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A novel procedure for the assessment of the antioxidant capacity of food components

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ABSTRACT

Carbonylation, an oxidative modification of the amino group of arginine and lysine residues caused by reactive oxygen species, has emerged as a new type of oxidative damage. Protein carbonylation has been shown to exert adverse effects on various protein functions. Recently, the role of food components in the attenuation of oxidative stress has been the focus of many studies. Most of these studies focused on the chemical properties of food components. However, it is also important to determine their effects on protein functions via post-translational modifications. In this study, we developed a novel procedure for evaluating the antioxidant capacity of food components. Hydrogen peroxide (H_2O_2)-induced protein carbonylation in HL-60 cells was quantitatively analyzed by using fluorescent dyes (Cy5–hydrazide dye and IC3–OSu dye), followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and fluorescence determination. Among a panel of food components tested, quinic acid, kaempferol, saponin, squalene, trigonelline, and mangiferin were shown to be capable of suppressing protein carbonylation in HL-60 cells. Our results demonstrated that this fluorescence labeling/SDS–PAGE procedure allows for the detection of oxidative stress-induced protein carbonylation with high sensitivity and quantitative accuracy. This method should be useful for the screening of new antioxidant food components as well as the analysis of their suppression mechanism.

Oxidative stress is defined as the disruption of the balance between oxidative damage by reactive oxygen species (ROS) and antioxidant capacity in vivo. ROS plays an important role in energy production, disinfection, and signal transduction [1–3]. Excess ROS, however, may disrupt biological processes by causing oxidative damage to lipids, proteins, enzymes, and DNA. Recently, protein carbonylation has been recognized as a post-translational modification induced by oxidative damage. Protein carbonylation, the conversion of the amine group of lysine, arginine, or proline residue to carbonyl derivatives (aldehyde or ketone) by ROS, has emerged as a negative regulator of protein functions [4–7]. This post-translational modification is an irreversible reaction. After carbonylation, proteins may lose their function in vivo and are degraded. It has been reported that carbonylated proteins accumulate with aging and in patients with diabetes, Alzheimer's disease, kidney failure, and arteriosclerosis [8–10]. Carbonylation is generally regarded as an oxidative modification marker. Thus, the analysis of carbonylated proteins by taking proteomic approaches might allow the identification of pathological changes induced by oxidative stress.

Recently, many studies have focused on the beneficial functions of food components, including antioxidative, anticancer, and hypotensive effects. In particular, food antioxidant capacity has received a great deal of attention. The antioxidant capacity of food components has mostly been analyzed using conventional methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) assays [11–13]. These studies focused exclusively on the ROS extinction capacity of food components. We recently embarked on examining the biological

Abbreviations used: ROS, reactive oxygen species; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ORAC, oxygen radical absorbance capacity; FBS, fetal bovine serum; H_2O_2, hydrogen peroxide; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; TCA, trichloroacetic acid; DCA, sodium deoxycholate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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activities of the antioxidants as well as their effects on protein function and gene expression. One particular aspect we were interested in is ROS-induced protein carbonylation. Our hypothesis is that protein carbonylation may reflect inversely the antioxidant capacity of food components. Protein carbonylation is commonly analyzed by immunoblotting using 2,4-dinitro phenylhydrazine (DNPH) [14,15]. However, this procedure is cumbersome, error-prone, and difficult to use in quantitative analysis.

In the current study, we took advantage of the reactivity of hydrazide groups with carbonyl groups. A highly sensitive, simple, and accurate hydrazide-containing fluorescence dye-based procedure was developed. Moreover, this new method was used to evaluate the antioxidant capacity of food components.

Materials and methods

Materials

RPMI 1640 medium and penicillin–streptomycin solution (×100) were products of Wako Pure Chemical (Osaka, Japan). Fetal bovine serum (FBS) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Cy5–hydrazide dye was purchased from Lumiprobe (Hallandale Beach, FL, USA). IC3–OSu dye was obtained from Dojindo Laboratories (Kumamoto, Japan). (*R*)-(+)-Limonene, saponin, sodium ascorbate, hydrogen peroxide (H_2O_2), and dimethyl sulfoxide (DMSO) were products of Wako Pure Chemical. Kaempferol, caffeine, and daidzein were obtained from Funakoshi (Tokyo, Japan). Trolox, all-trans-astaxanthin, galangin, squalene, mangiferin, and trigonelline hydrochloride were products of Sigma-Aldrich (St. Louis, MO, USA). *D*-(−)-Quinic acid was obtained from Alfa Aesar (Heysham, Lancashire, UK).

Cell culture

HL-60 cells (JCRB0085) were obtained from JCRB Cell Bank (Osaka, Japan). HL-60 cells were cultured in RPMI 1640 medium containing 10% heat-inactivated FBS and penicillin–streptomycin (100 IU/ml and 50 μg/ml, respectively) and maintained at 37 °C in a humid atmosphere containing 95% air and 5% CO_2.

Cell viability assay

Cell viability was assayed by the trypan blue dye exclusion method. After incubating the cells with various concentrations of H_2O_2 (0, 5, 10, 20, 40, 60, 80, and 100 μM) for 4 h, the culture medium was removed and the cells were resuspended with phosphate-buffered saline (PBS). Then 500 μl of 0.4% trypan blue was added to 500 μl of the cell suspension in the wells of a 6-well plate. The viable cells were counted by using an inverted microscope.

Protein carbonylation of HL-60 cells treated with H_2O_2

A 1-mM stock solution of H_2O_2 was freshly prepared prior to each experiment. A total of 10,000 HL-60 cells were seeded per well in a 6-well plate. For ex vivo carbonylation, HL-60 cells were exposed to various concentrations of H_2O_2 (0, 1, 5, 10, 20, and 40 μM) in FBS–RPMI 1640 and incubated at 37 °C for 4 h.

Determination of antioxidant capacity of food components by measuring protein carbonylation

Antioxidant capacity of various food components was evaluated by measuring protein carbonylation with sodium ascorbate and trolox as positive controls. A total of 10,000 HL-60 cells per well were seeded in individual wells of a 6–well plate. HL-60 cells in individual wells were exposed to various concentrations of a tested food component or, as positive control, sodium ascorbate and trolox (0, 20, 40, 100, and 200 μM) in RPMI 1640 medium at 37 °C for 24 h, followed by incubation for 4 h with 40 μM H_2O_2 at 37 °C. At the end of the incubation, the supernatants were removed and

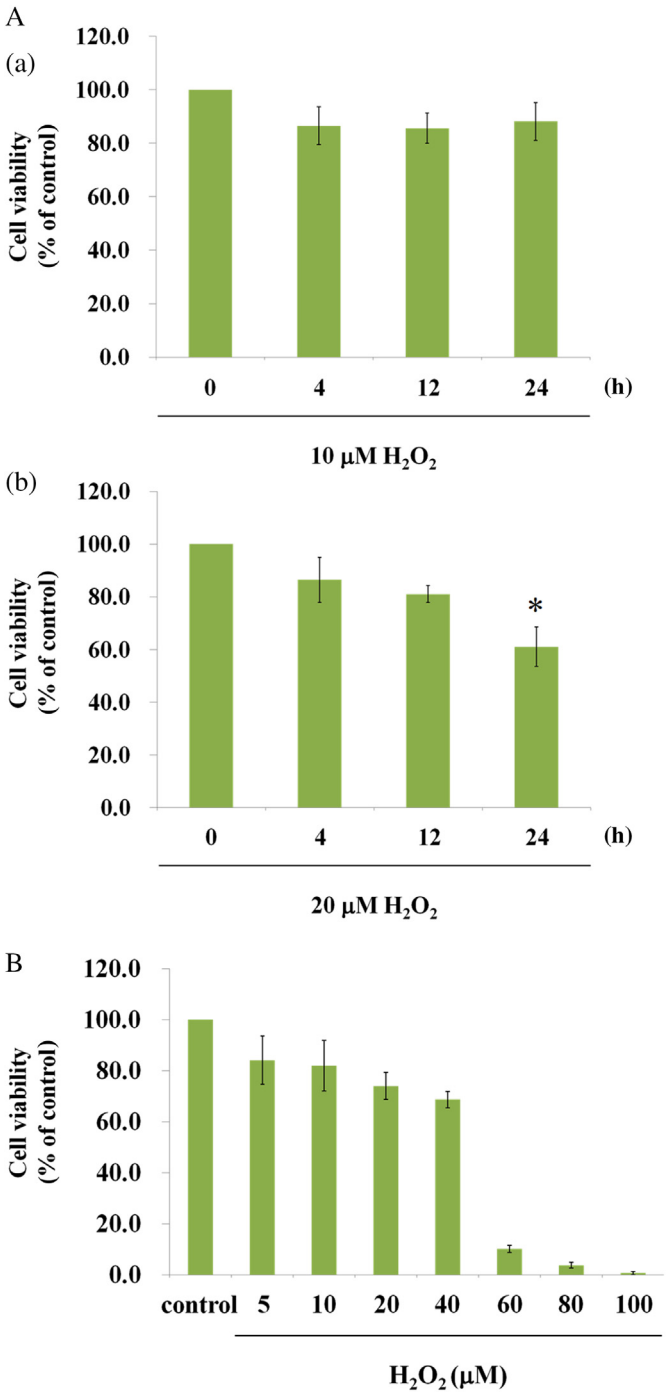

Fig.1. Effects of H_2O_2 on cell viability. (A) Time-dependent effects of H_2O_2 on the viability of HL-60 cells. Panel (a) indicates cell viability after a 24-h treatment with 10 μM H_2O_2 at 37 °C. Panel (b) indicates cell viability after a 24-h treatment with 20 μM H_2O_2 at 37 °C. (B) Concentration-dependent effects of H_2O_2 on the viability of HL-60 cells. Data shown represent means ± standard deviations derived from three determinations. Significant difference versus each control: *$P < 0.05$ (Dunnett's test).