Amplification of Antioxidant Activity of Catechin by Polycondensation with Acetaldehyde

Joo Eun Chung,[†,‡] Motoichi Kurisawa,[†,‡] Young-Jin Kim,[†] Hiroshi Uyama,*[,†] and Shiro Kobayashi*[,†]

Department of Materials Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, 615-8510, Japan, Bio-oriented Technology Research Advancement Institution

Received July 17, 2003; Revised Manuscript Received September 12, 2003

Catechin exhibits numerous biological and pharmacological effects attributed to antioxidant action. The synthetic poly(catechin)s condensed through acetaldehyde with different molecular weights were assessed in terms of antioxidant activity and enzyme inhibitory activity on the basis of a catechin repeating unit and compared with monomeric catechin. The poly(catechin)s showed great amplification of superoxide scavenging activity, xanthine oxidase (XO) inhibitory activity, and inhibition effects on human low-density lipoprotein oxidation initiated by 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) as a radical generator on the catechin unit level, compared to monomeric catechin: these activities were proportional to their molecular weights. The reducing power of the polymer was lower than that of monomeric catechin, which decreased with increasing the molecular weight. The polymer also protected endothelial cells from oxidative injury induced by AAPH, with a greater effect expressed on a catechin unit basis than that of the monomer. These results demonstrate that the poly(catechin)s are more potent antioxidant agents and enzyme inhibitors.

Introduction

Catechin, an ingredient of green tea and wine, belongs to the flavonoids which are one of the most numerous and best-studied groups of plant polyphenols. The flavonoids consist of a large group of low-molecular weight polyphenolic substances, naturally occurring in fruits and vegetables, and are an integral part of the human diet. Their biological and pharmacological effects including antioxidant, anti-mutagenic, anti-carcinogenic, antiviral, and anti-inflammatory properties, have been demonstrated in numerous human, animal, and in vitro studies.[1−3] These properties are potentially beneficial in preventing diseases and protecting the stability of the genome. Many of these activities have been related to its antioxidant actions.[4−6]

In general, the activities of flavonoids are known to be limited for only few hours in a body, although the metabolism has not been established. In addition, several flavonoids have been shown to act as pro-oxidants and generate reactive oxygen species, such as hydrogen peroxide.[7−9] In contrast, a relatively high-molecular fraction of extracted plant polyphenols has been reported to exhibit enhanced physiological properties such as antioxidant and anti-carcinogenic activity, and a relatively longer circulation time in vivo.[6,10−13] High molecular weight plant polyphenols have also been reported to show no pro-oxidant effects.[12,14] Many investigations have explored the antioxidant effects of low-molecular weight flavonoids but few have considered the polymers of flavonoids: most of them have concentrated on condensed tannins (proanthocyanidins), a high-molecular fraction of extracted polyphenols from natural plants. We have synthetically designed the polymers of flavonoids and polymer-flavonoid conjugates, with consideration to the extension of the amplification of physiological properties of the flavonoids.[15−17]

Among naturally occurring polymers of flavonoids, particularly, the polymerized compounds in red wines have attracted much attention in relation to their potential physiological activities. The compounds, responsible for a part of the organoleptic properties of red wines, are progressively formed as a result from the auto-polymerization between flavan-3-ol units such as catechins or between flavan-3-ol and anthocyanin units, by either of two main processes: a direct condensation or an indirect condensation with acetaldehyde during conservation and aging of red wines.[18−24] As a representative direct condensate, proanthocyanidins, one of the main constituents of red wines, have been extensively studied. They are polymers with a structure consisting of (+)-catechin and (−)-epicatechin units directly linked by C_4-C_6 or C_4-C_8 bonds and sometimes esterified by gallic acid on the epicatechin moieties.[25] Several investigations for a potent antioxidant activity of proanthocyanidins reported that the ability to scavenge free radicals was correlated with the degree of polymerization.[6,10,26] However, it was contradicted by the opposite results that the scavenging ability was not proportional relationship to the degree of polymerization when expressed by monomeric unit equivalents, and rather the ability increased with an increase in the number of gallic acid moieties which simultaneously raised with the degree of polymerization in the molecule.[27,28]

* To whom correspondence should be addressed. Phone: +81-75-383-2460; +81-75-383-2459. Fax: +81-75-383-2461. E-mail: uyama@mat.polym.kyoto-u.ac.jp; kobayasi@mat.polym.kyoto-u.ac.jp.

† Kyoto University.

‡ Bio-oriented Technology Research Advancement Institution.

10.1021/bm0342436 CCC: $27.50
Published on Web 11/01/2003

On the other hand, indirect condensation with acetaldehyde produced in wine by yeasts during wine-making and also by oxidation of ethanol during aging[29] could concern between flavan-3-ol units or between flavan-3-ol and anthocyanin units. Whereas this type of condensation reaction has been confirmed by many researchers, the physiological activity of the produced polymers has not been studied.

We focused our attention on the poly(catechin)s condensed through acetaldehyde as one of the synthetic approaches to the potent strategic molecular designs for extension of the amplification of physiological properties of catechin. In the present study, the poly(catechin)s with different molecular weights were chosen to explore antioxidant activity represented by the activities to scavenge superoxide radicals, to inhibit peroxidation of human low-density lipoprotein (LDL) as an in vitro pathogenic model for atherogenesis, and to protect cultured endothelial cells from oxidative injury by free radicals. In addition, enzyme (xanthine oxidase) inhibitory activity of the polymers was investigated. We noticed that in most cases physiological activity of flavonoid polymers has been compared to the corresponding monomeric flavonoid on their molar basis. We tried, herein, to evaluate the physiological activity of the poly(catechin)s on the basis of the monomeric repeating unit, to make comparisons based on the activity of a single catechin unit in the polymer molecules with a monomeric catechin.

Experimental Section

Materials. (+)-Catechin and acetaldehyde were purchased from Tokyo Kasei Co., Japan. Low-density lipoprotein (LDL) from human plasma, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Sigma. Xanthine, xanthine oxidase (from butter milk), 2,2′-azobis(2-amidinopropane)-dihydrochloride (AAPH), dibutyl hydroxytoluene (BHT), nitroblue tetrazolium (NBT), vitamin C, Trolox, and ethylenediamine tetraacetic acid (EDTA) were obtained from Wako Pure Chemical Industries, Japan. Diphenyl-1-pyrenylphosphine (DPPP) was purchased from Dojindo, Japan. Bovine aortic endothelial cells were purchased from Dainippon Pharmaceutical Co., Ltd., Japan. Alamar blue was purchased from Trek Diagnostic Systems Ltd., U.K. Other reagents and solvents are commercially available and used as received.

Synthesis of Poly(catechin)s. Poly(catechin)s were synthesized by the little modified method reported by Fulcrand et al.[23] (+)-Catechin (4.7×10^{-2} M) was dissolved in a mixture of acetic acid (4.1 mL), ethanol (12.1 mL), and water (90.1 mL). The reaction was started by addition of acetaldehyde at different concentration levels (2.4 and 4.7×10^{-2} M) and performed at 35 °C under air. The resulting products were separated by centrifugation and washing with a mixture of ethanol and water in three replicates, followed by drying in vacuo to give the polymers. The molecular weight was estimated by size exclusion chromatography (SEC, Tosoh GPC-8020 equipped with RI-8020 detector) with two TSKgel α-M columns using DMF containing 0.10 M LiCl as eluent, after acetylation.[30,31] ^{1}H and ^{13}C NMR were recorded on a Bruker DPX400 spectrometer.

^{1}H NMR (DMSO-d_6): δ 1.1−1.8 (CHCH_3), 2.3−3.1 (H-4 of C ring), 3.6−4.1 (H-3 of C ring), 4.3−4.6 (H-2 of C ring), 4.6−4.9 (CHCH$_3$), 6.4−6.9 (H-2′, 5′, and 6′ of B ring).

^{13}C NMR (DMSO-d_6): δ 18−21 (CHCH$_3$), 21−24 (CHCH$_3$), 27−31 (C-4 of C ring), 68−70 (C-3 of C-ring), 82−85 (C-2 of C ring), 101−105 (C-4a of A-ring), 106−109 (C-8 of A ring), 112−115 (C-6 of A ring), 116−118 (C-2′ and 5′ of B-ring), 120−122 (C-6′ of B-ring), 132−134 (C-1′ of B ring), 146−148 (C-3′ and 4′ of B-ring), 151−156 (C-5, 7, and 8a of A-ring).

Superoxide Radical Scavenging Activity. The superoxide radical was generated by xanthine/xanthine oxidase (XO) and measured by the nitroblue tetrazolium (NBT) reduction method.[32] A test sample was mixed in a 100 mM phosphate buffer solution (pH 7.0) containing XO (1.65×10^{-2} units mL^{-1}) and NBT (133 μM) at 25 °C in 96-well flat-bottomed microassay plates. The measurement was started with adding xanthine (164 μM). Production of superoxide radical was followed spectrophotometrically at 560 nm at 25 °C for 10 min using a Synergy HT Microplate Photoncounter (BIO-TEK Instruments, Inc., U.S.A.). All analyses were run in three replicates and averaged. Superoxide scavenging activity was calculated according to the following formula:

$$\text{superoxide scavenging activity (\%)} = \frac{\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}} \times 100$$

where absorbance$_{\text{control}}$ and absorbance$_{\text{sample}}$ represent the increased absorbance in the absence and presence of samples, respectively.

Evaluation of the Reducing Power. The reducing power of samples was determined according to the method of Oyaizu.[33] Samples (5−40 μM) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%); the mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

XO Inhibitory Activity. The activity of XO was measured spectrophotometrically by monitoring the formation of uric acid from xanthine at 295 nm for 30 min by a UV−visible spectrometer (Hitachi U-2001, Japan).[34] The assay was carried out at a same condition as that of superoxide radical assay mentioned above, and the percentage of activity was calculated.

Determination of Oxidized LDL Phospholipids. Immediately before oxidation, human low-density lipoprotein (LDL) was dialyzed against 1000 times the volume of the degassed 10 mM phosphate buffered saline solution (pH 7.4) containing 2.7 mM KCl and 0.137 M NaCl in the dark for 24 h at 4 °C with continuous nitrogen sparging. The dialysis solution was changed four times. LDL (100 μg mL^{-1}) was incubated with 200 μM (final concentration) of diphenyl-1-pyrenylphosphine (DPPP) for 5 min at 37 °C in the dark

under N_2. Before LDL oxidation, the DPPP-labeled LDL was preincubated in the absence or presence of various concentrations (0−80 μM) of a sample for 30 min at 37 °C in the dark. Oxidation of LDL preincubated in the absence or presence of a sample was carried out by further incubation with AAPH (1mM) for 1 h at 37 °C. Oxidation of DPPP was measured using a 1420 ARVOSX multilabel counter (Wallac, Perkin-Elmer). Wavelengths of excitation and emission were set at 355 and 405 nm, respectively.

Determination of Oxidized Apo B Formation. Dialyzed LDL (100 μg mL^{-1}) was preincubated in the absence or presence of a sample (8.8 μM) for 30 min at 37 °C in the dark. The LDL was oxidized by further incubation with AAPH (1 mM) for 1 h at 37 °C. The extent of aldehyde-modified lysine in oxidized LDL was observed using an excitation wavelength of 350 nm and monitoring emission fluorescence (390−470 nm) with a fluorescence spectrophotometer (Hitachi F-2500, Japan).

Cell Culture. Bovine aortic endothelial cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/mL of penicillin, and 100 μg/mL of streptomycin in a humidified atmosphere 5% CO_2 incubator.

Evaluation of Protection Effects on Radical-Induced Cytotoxicity. Bovine endothelial cells were seeded at a concentration of 2×10^4 cells mL^{-1}, 200 μL well^{-1}, in 96-well flat-bottomed microassay plates (Falcon 3072 Co., Becton Dickenson, Flanklin Lakes, NJ) for 24 h before adding sample solutions. A desired amount of sample stock solutions was diluted into DMEM without FBS. The sample solution (190 μL) was added into 96-well plates and preincubated for 1 h at 37 °C. The AAPH solution (10 μL, 200 mM) as a radical generator was added into the well, and the cells were cultured for 48 h at 37 °C in a humidified 5% CO_2 atmosphere. To evaluate of cell viability, 22 μL of alamar blue, a dye which changes color from blue to red when subjected to reduction by cytochrome *c* activity, was added to culture media and incubated for 4 h at 37 °C.[35] The optical absorbance was read at 560 and 600 nm to obtain a dye reduction amount using a 1420 ARVOSX multilabel counter (Wallac).

Results and Discussion

Poly(catechin) Condensed through Acetaldehyde. In this study, poly(catechin)s with different molecular weights, M_n = 2760 (M_w/M_n = 2.1) and 890 (M_w/M_n = 1.2) (PC-1 and PC-2, respectively) were successfully synthesized in high yields by changing the concentration of acetaldehyde. ^{1}H and ^{13}C NMR analysis of the product revealed that condensation of (+)-catechin in the presence of acetaldehyde gave poly(catechin)s with the structure composed of catechin units linked at the C_6 and C_8 positions of the A ring (Figure 1). Several studies have shown the similar results that flavanols can be linked through C_6-C_6, C_8-C_8, and C_6-C_8 (R and S) bonds, taking into account the presence of an asymmetric carbon for the C_6-C_8 isomers.[20,22−24]

Superoxide Radical Scavenging Activity. The superoxide radical is a reactive oxygen species, which is formed during normal aerobic metabolism and by activated phagocytes.[36] Reduction of molecular oxygen to superoxide by xanthine oxidase (XO), generating hydroxyl radicals and uric acid, is an important physiological pathway.[37] However, an excess of superoxide radicals damages biomacromolecules both directly and indirectly by forming hydrogen peroxide or highly reactive hydroxyl radicals.[38]

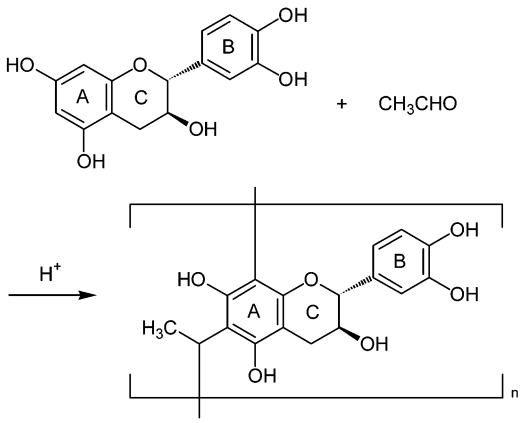

Figure 1. Synthesis of poly(catechin) condensed through acetaldehyde.

Table 1. Free Radical Scavenging Activity of the Poly(catechin)s, $n = 3$

sample	superoxide scavenging activity IC_{50} (μM)
PC-1	3.5 ± 0.4[a]
PC-2	4.0 ± 0.2[a]
catechin	18.0 ± 0.6
vitamin C	18.8 ± 0.5
BHT	≫200

[a] Concentrations of catechin repeating units.

A mixture of xanthine and XO generates superoxide radicals, which reduces nitroblue tetrazolium (NBT) to give the blue chromogen formazan and increases UV absorbance at 560 nm.[33] Compounds capable of scavenging superoxide radicals, such as superoxide dismutase (SOD), inhibit NBT reduction. The antioxidant activity of poly(catechin)s with different molecular weights was evaluated in terms of the superoxide radical scavenging activity (Table 1). We found that the poly(catechin)s showed the markedly higher SOD-like activity on the basis of a monomeric repeating unit, with quite lower IC_{50} (the concentration of monomeric units needed to scavenge superoxide radical by 50%), compared to the catechin monomer, vitamin C and dibutyl hydroxytoluene (BHT). The scavenging activity of poly(catechin)s was dependent on the molecular weight: PC-1 exhibited the greatest scavenging activity with less than 1/5 of the IC_{50} observed for monomeric catechin. A control experiment revealed that the samples did not directly reduce NBT in the range of concentrations tested. These results infer that a single constituent catechin unit of the poly(catechin) has a more potent scavenging activity against the superoxide radical than that of a monomeric catechin. We have preliminarily observed the consistent phenomena for enzymatically synthesized polymers of catechin.[15]

Reducing Power. Although the above poly(catechin)s showed markedly amplified antioxidant activity with increas-

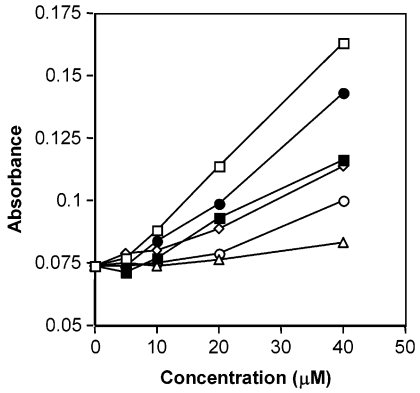

Figure 2. Reducing power of the poly(catechin)s, $n = 3$. ■, PC-1; ●, PC-2; □, catechin; ○, vitamin C; △, BHT; and ◇, Trolox.

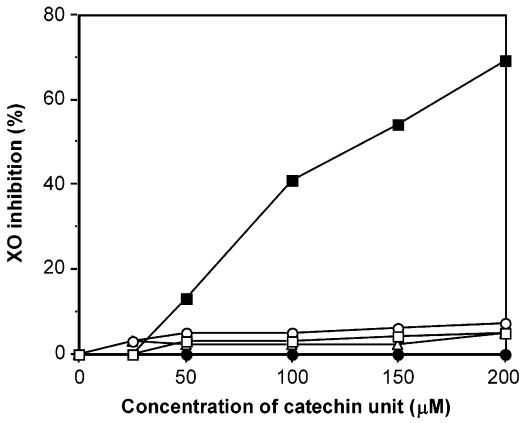

Figure 3. XO inhibition activity of the poly(catechin)s, $n = 3$. ■, PC-1; ●, PC-2; □, catechin; ○, vitamin C; and △, BHT.

ing molecular weight, their reducing power was lower as the molecular weight is higher, designating worse reducing agents than monomeric catechin (Figure 2). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[39] However, there is not always a linear correlation between the reducing capacity and total antioxidant activity.[40] Furthermore, the antioxidant activity of putative antioxidants has been attributed to various mechanisms which include prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, radical scavenging, etc.[41]

The functional group responsible for each redox potential was identified by cyclic voltammetry of monomeric phenolic compounds.[42] These redox potentials of the monomers were found to correspond to each functional group of the polymeric phenolics constituted by the similar monomeric phenolics. For the polymers of phenolics, the redox potentials have been demonstrated to be equivalent to or slightly lower than the corresponding monomeric phenolics.[12] Hagerman et al. demonstrated that high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for free radical scavenging by the polymeric phenolics than specific functional groups.[12]

Our results suggest that the amplified antioxidant activity of the poly(catechin)s might be contributed to by the creation of an extremely high proximity of phenolic moieties in a molecule developing the activity to scavenge free radicals rather than the reducing power.

Xanthine Oxidase Inhibitory Activity. XO is not only an important biological source of reactive oxygen species but also the enzyme responsible for the formation of uric acid associated with gout leading to painful inflammation in the joints.[43,44] Figure 3 showed XO inhibitory activity assessed by evaluating uric acid formation from XO.[34] The XO inhibition effects of samples were negligible at a concentration below 200 μM, with the single exception of PC-1 exhibiting an increase in XO inhibitory activity as an increasing concentration of repeating catechin units. This markedly amplified XO inhibitory activity of PC-1 was considered to be due to effective multivalent interaction between XO and the condensed catechin units in the effective polymeric chain,[15] only when the molecular weight is high enough. Compounds capable of inhibiting XO can also positively affect the activity to scavenge superoxide radicals. However, XO inhibitory activities of the poly(catechin)s were negligible in the range of concentrations tested in Table. 1. Thus, the amplified scavenging activity of the poly(catechin)s resulted primarily from an increase in scavenging activity against superoxide radicals, rather than in the inhibitory effect on XO.

Inhibition against LDL Peroxidation. A good example of the significance of oxidative modification of protein in vivo has been reported for human LDL, where modification by oxidation was associated with the pathogenic process of atherogenesis.[45] Peroxidation of LDL leads to its enhanced uptake by macrophages, which is believed subsequently to result in foam cell formation, one of the first stages of atherogenesis. Therefore, antioxidants that protect LDL against oxidation are potentially anti-atherogenic compounds. Although the mechanism for in vivo oxidation of LDL has not been established, free radical auto-oxidation may be a factor. To evaluate the antioxidant effect against the peroxidation of LDL, LDL was labeled with diphenyl-1-pyrenylphosphine (DPPP), a fluorescent probe sensing hydroperoxide produced by lipid oxidation. The labeled LDL was preincubated with a sample of antioxidant, prior to oxidation by addition of 2,2′-azobis(2-amidinopropane)-dihydrochloride (AAPH), a radical generator. Incubation of AAPH with LDL generates peroxyl radicals, leading to a chain reaction which gives peroxidation products such as hydroperoxides and aldehydes.[46] DPPP, a nonfluorescent molecule, reacts stoichiometrically with hydroperoxide to give diphenyl-1-pyrenylphosphine oxide (DPPP═O), which is strongly fluorescent.[47] Poly(catechin)s showed greater inhibitory activities against LDL peroxidation in a catechin unit-concentration dependent manner, compared to monomeric catechin (Figure 4). The amplification of inhibitory activity was greater, as the molecular weight was higher. These data suggest that a structure of poly(catechin) is much more capable of inhibiting peroxidation of LDL than that of the monomer.

When LDL phospholipid undergoes oxidation by peroxyl radicals, reactive aldehydes are formed that bind to apolipoprotein B (apo B), the major protein component of LDL. Oxidized LDL showed the fluorescence properties attributed to the formation of Schiff base products between reactive

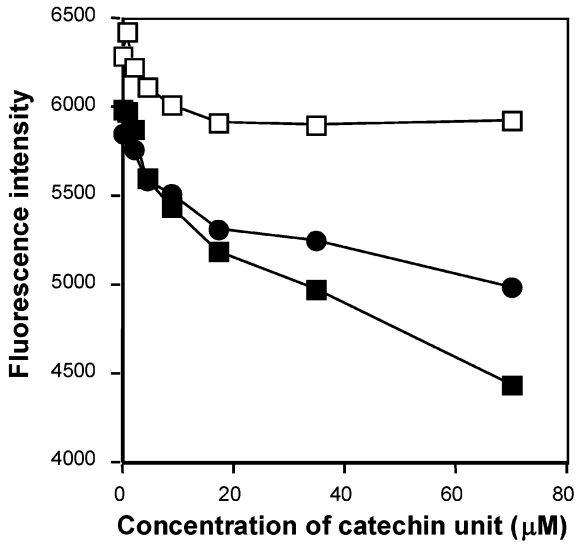

Figure 4. Inhibition effect of the poly(catechin)s against phospholipid oxidation of LDL induced by AAPH. ■, LDL plus initiator plus PC-1; ●, LDL plus initiator plus PC-2; and □, LDL plus initiator plus catechin; [catechin unit] = 8.8 μM, $n = 8$.

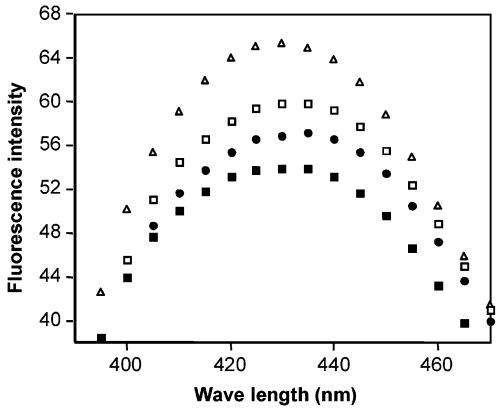

Figure 5. Inhibition effect of the poly(catechin)s against oxidized apo B formation in LDL induced by AAPH. △, LDL plus AAPH; ■, LDL plus AAPH plus PC-1; ●, LDL plus AAPH plus PC-2; and □, LDL plus AAPH plus catechin; [catechin unit] = 8.8 μM.

aldehydes and lysine residues of apo B.[48] The poly(catechin)s more effectively inhibited modified-apo B formation induced by AAPH, with a proportional relationship to their molecular weight, compared to monomeric catechin (Figure 5). The samples themselves did not quench the fluorescence when added to an LDL solution that was already oxidatively modified. These results imply that the poly(catechin) is a powerful chain-breaking antioxidant, which possesses a much higher potential for protecting LDL against both phospholipid peroxidation and apo B modification, induced by free radicals than does monomeric catechin.

Inhibition of Radical-Induced Cytotoxicity. Reactive radical species result in various cell damage including oxidative deterioration of lipids, protein, and DNA, inducing cell death such as apoptosis and necrosis.[49] Protection effects of catechin and the poly(catechin) against endothelial cell damage caused by AAPH were examined (Figure 6). AAPH induces peroxidation only in the lipid membrane of cells during the first step.[50] The addition of AAPH caused cell death due to oxidative injury. However, the poly(catechin) (PC-1) enhanced cell viability with higher protection effects

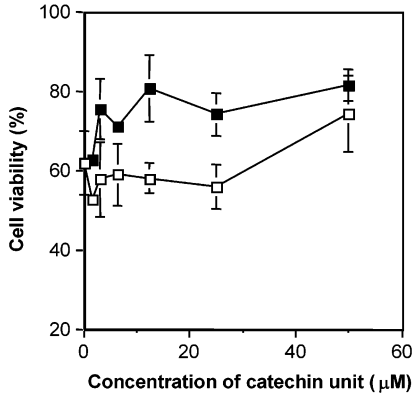

Figure 6. Protection effects of the poly(catechin) against cell injury induced by AAPH. ■, PC-1 and □, catechin, $n = 8$.

against the oxidative damage than that of the monomeric catechin which was ineffective below 25 μM.

Polymers of flavonoids occurring in nature, mainly proanthocyanidin, have retained the attention in recent years due to their effective antioxidant activity and no-prooxidant activity. Although the ability to scavenge peroxyl radicals trends to be proportional to the degree of polymerization,[6,10,25] the mechanism has not been established. It has been reported that the hydroxyl groups of flavonoids are closely related to their physiological activities: for example, the flavonoids with the most hydroxyl groups were most easily oxidized.[51] Several studies demonstrated that the presence of a catechol group on the B-ring of flavonoids contributed to chain-breaking antioxidant activity and conferred higher stability to the radical form in electron delocalization.[52,53] Taking account of an abundance of condensed catechol groups on the B-ring in a molecule of flavonoid polymers, the effective antioxidant activity in molar basis reported for tannins would come up to the expectation. On the other hand, the present poly(catechin)s have proved that the antioxidant activity and the enzyme−inhibitory activity were amplified on the basis of a constituent catechin unit, correlating to the molecular weight. We have found that the poly(catechin)s are constructed by regular helical structures through intramolecular hydrogen bonds, only when the chain is relatively long (data not shown). The structure of high-ordered catechin units might be one contribution responsible for the amplification of either antioxidant activity or enzyme inhibitory activity (interaction with proteins) on the catechin unit level.

Conclusion

The present poly(catechin)s were much more potent antioxidant agents and enzyme inhibitors than monomeric catechin. They showed great amplification of the activities to scavenge superoxide radicals, to inhibit XO and peroxidation of LDL, and to protect endothelial cells from oxidative injury. These activities increased in correlation to the molecular weight. The reducing power of the polymers was lower than that of the monomer with a reverse relationship to the molecular weight. We expect that poly(catechin) is useful for a therapeutic agent to offer protection against a wide range of free radical-induced and/or enzyme-related diseases including cardiovascular diseases, atherogenesis, gout and cancer.

Acknowledgment. This work was partly supported by Program for Promotion of Basic Research Activities for Innovative Bioscience. We are grateful to Professor Yasuhiro Aoyama for the use of the fluorescence spectrometer.

References and Notes

(1) Jankun, J.; Selman, S. H.; Swiercz, R.; Skrzypczak-Jankun, E. *Nature* **1997**, *387*, 561.
(2) Bordoni, A.; Hrelia, S.; Angeloni, C.; Giordano, E.; Guarnieri, C.; Caldarera, C. M.; Biagi, P. L. *J. Nutr. Biochem.* **2002**, *3*, 103.
(3) Nakagawa, K.; Ninomiya, M.; Okubo, T.; Aoi, N.; Juneja, L. R.; Kim, M.; Yamanaka, K.; Miyazawa, T. *J. Agric. Food Chem.* **1999**, *47*, 3967.
(4) Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. *J. Am. Chem. Soc.* **1994**, *116*, 4846.
(5) Yen, G. C.; Chen, H. Y. *J. Agric. Food Chem.* **1995**, *43*, 27.
(6) Zhao, J.; Wang, J.; Chen, Y.; Agarwal, R. *Carcinogenesis* **1999**, *20*, 1737.
(7) Yen, G. C.; Chen, H. Y.; Peng, H. H. *J. Agric. Food Chem.* **1997**, *45*, 30.
(8) Yamanaka, N.; Oda, O.; Nagao, S. *FEBS Lett.* **1997**, *401*, 230.
(9) Roedig-Penman, A.; Gordon, M. H. *J. Agric. Food Chem.* **1997**, *45*, 4267.
(10) Ariga, T.; Hamano, M. *Agric. Biol. Chem.* **1990**, *54*, 2499.
(11) Saito, M.; Hosoyama, H.; Ariga, T.; Kataoka, S.; Yamaji, N. *J. Agric. Food Chem.* **1998**, *46*, 1460.
(12) Hagerman, A. E.; Riedl, K. M.; Jones, G. A.; Sovik, K. N.; Ritchard, N. T.; Hartzfeld, P. W.; Riechel, T. L. *J. Agric. Food Chem.* **1998**, *46*, 1887.
(13) Duweler, K. G.; Rohdewald, P. *Pharmazie* **2000**, *55*, 364.
(14) Li, C.; Xie, B. *J. Agric. Food Chem.* **2000**, *48*, 6362.
(15) Kurisawa, M.; Chung, J. E.; Kim, Y. J.; Uyama, H.; Kobayashi, S. *Biomacromolecules* **2003**, *4*, 469.
(16) Chung, J. E.; Kurisawa, M.; Tachibana, Y.; Uyama, H.; Kobayashi, S. *Chem. Lett.* **2003**, *32*, 620.
(17) Kurisawa, M.; Chung, J. E.; Uyama, H.; Kobayashi, S. *Biomacromolecules* **2003**, *4*, 1394.
(18) Liao, H.; Cai, Y.; Haslam, E. *J. Sci. Food Agric.* **1992**, *59*, 299.
(19) Santos-Buelga, C.; Bravo-Haro, S.; Rivas-Gonzalo, J. C. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 269.
(20) Saucier, C.; Bourgeois, G.; Vitry, C.; Roux, D.; Glories, Y. *J. Agric. Food Chem.* **1997**, *45*, 1045.
(21) Es−Safi, N. E.; Fulcrand, H.; Cheynier, V.; Moutounet, M. *J. Agric. Food Chem.* **1999**, *47*, 2088.
(22) Es-Safi, N.; Fulcrand, H.; Cheynier, V.; Moutounet, M.: Hmanmouchi, M.; Essassi, E. M. In *Polyphenols Communications 96*; Vercauteren, J., Cheze, C., Cumon, M. C., Weber, J. F., Eds.; Groupe Polyphenols: Bordeauz, 1996.
(23) Fulcrand, H.; Doco, T.; Es-Safi, N. E.; Cheynier, V.; Moutounet, M. *J. Chromatogr. A* **1996**, *752*, 85.
(24) Saucier, C.; Guerra, C.; Pianet, I.; Laguerre, M.; Glories, Y. *Phytochemistry* **1997**, *46*, 229.
(25) Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. *Phytochemistry* **1994**, *36*, 781.
(26) Uchida, S.; Edamatsu, R.; Hiramatsu, M.; Mori, A.; Nonaka, G.; Nishioka, Y. I.; Niwa, M.; Ozaki, M. *Med. Sci. Res.* **1987**, *15*, 831.
(27) Jorge, M.; Silva, R.; Darmon, N.; Fernandez, Y.; Mitjavila, S. *J. Agric. Food Chem.* **1991**, *39*, 1549.
(28) Arteel, G. E.; Sies, H. *FEBS Lett.* **1999**, *462*, 167.
(29) Romano, P.; Suzzi, G.; Turbanti, L.; Polsinelli, M. *FEMS Microbiol. Lett.* **1994**, *118*, 213.
(30) Mita, N.; Maruichi, N.; Tonami, H.; Nagahata, R.; Tawaki, S.; Uyama, H.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 375.
(31) Williams, V. M.; Porter, L. J.; Hemingway, R. W. *Phytochemistry* **1983**, *22*, 569.
(32) Rajakumar, D. V.; Rao, M. N. *Biochem. Pharmacol.* **1993**, *46*, 2067.
(33) Oyaizu, M. *Jpn. J. Natr.* **1986**, *44*, 307.
(34) Noro, T.; Oda, Y.; Miyase, T.; Ueno, A.; Fukushima, S. *Chem. Pharm. Bull.* **1983**, *31*, 3984.
(35) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
(36) Fantone, J. C.; Ward, P. A. *Hum. Pathol.* **1985**, *16*, 973.
(37) Halliwell, B. In *Free Radicals in Biology and Medicine*; Gutteridge, J. M. C., Ed.; Clarendon Press: Oxford, U.K., 1989.
(38) Floyd, R. A. *FASEB J.* **1990**, *4*, 2587.
(39) Meir, S.; Kanner, J.; Akiri, B.; Hadas, S. P. *J. Agric. Food Chem.* **1995**, *43*, 1813.
(40) Yildirim, A.; Mavi, A.; Oktay, M.; Kara, A. A.; Algur, O. F.; Bilaloglu, V. *J. Agric. Food Chem.* **2000**, *48*, 5030.
(41) Diplock, A. T. *Free Radical Res.* **1997**, *27*, 511.
(42) Lunte, S. M.; Blankenship, K. D.; Read, S. A. *Analyst.* **1988**, *113*, 99.
(43) McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1968**, *243*, 5753.
(44) Chiang, H. C.; Lo, Y. J.; Lu, F. J. *J. Enzyme Inhib.* **1994**, *8*, 61.
(45) Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. *N. Engl. J. Med.* **1989**, *320*, 915.
(46) Niki, E. *Methods Enzymol.* **1990**, *186*, 100.
(47) Akasaka, K.; Suzuki, T.; Ohrui, H.; Meguro, H. *Anal. Lett.* **1987**, *20*, 797.
(48) Esterbauer, H.; Schaur, R. J.; Zollner, H. *Free Radical Biol. Med.* **1991**, *11*, 81.
(49) Halliwell, B. *Lancet* **1994**, *344*, 721.
(50) Takahashi, M.; Shibata, M.; Niki, E. *Free Radical Biol. Med.* **2001**, *31*, 164.
(51) Hodnick, W. F.; Milosavljevic, E. B.; Nelson, J. H.; Pardini, R. S. *Pharmacology* **1988**, *37*, 2607.
(52) Salah, N.; Miller, N. J.; Paganga, G.; Tijburg, L.; Bolwell, G. P.; Rice-Evans, C. *Arch. Biochem. Biophys.* **1995**, *322*, 339.
(53) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Radical Biol. Med.* **1996**, *20*, 933.

BM0342436