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Research paper

Effect of electron donating groups on polyphenol-based antioxidant dendrimers

Choon Young Lee [a, *], Cyprien N. Nanah [a], Rich A. Held [a], Amanda R. Clark [a], Uyen G.T. Huynh [a], Marina C. Maraskine [a], Rebecca L. Uzarski [b], John McCracken [c], Ajit Sharma [a, **]

[a] Department of Chemistry, Central Michigan University, Mount Pleasant, MI 48859, USA
[b] Department of Biology, Central Michigan University, Mount Pleasant, MI 48859, USA
[c] Department of Chemistry, Michigan State University, East Lansing, MI 48824, USA

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ABSTRACT

Numerous studies have reported the beneficial effects of antioxidants in human diseases. Among their biological effects, a majority of antioxidants scavenge reactive radicals in the body, thereby reducing oxidative stress that is associated with the pathogenesis of many diseases. Antioxidant dendrimers are a new class of potent antioxidant compounds reported recently. In this study, six polyphenol-based antioxidant dendrimers with or without electron donating groups (methoxy group) were synthesized in order to elucidate the influence of electron donating groups (EDG) on their antioxidant activities. Syringaldehyde (2 *ortho* methoxy groups), vanillin (1 *ortho* methoxy group), and 4-hydroxybenzaldehyde (0 methoxy group) were derivatized with propargylamine to form building blocks for the dendrimers. All the six dendrimers contain polyether cores, which were synthesized by attaching pentaerythritol and methyl α-D-glucopyranoside to in-house prepared spacer units. To prepare generation 1 antioxidant dendrimers, microwave energy and granulated metallic copper catalyst were used to link the cores and building blocks together via alkyne-azide 1,3-cycloaddition click chemistry. These reaction conditions resulted in high yields of the target dendrimers that were free from copper contamination. Based on DPPH antioxidant assay, antioxidant dendrimers decorated with syringaldehyde and vanillin exhibited over 70- and 170-fold increase in antioxidant activity compared to syringaldehyde and vanillin, respectively. The antioxidant activity of dendrimers increased with increasing number of EDG groups. Similar results were obtained when the dendrimers were used to protect DNA and human LDL against organic carbon and nitrogen-based free radicals. In addition, the antioxidant dendrimers did not show any pro-oxidant activity on DNA in the presence of physiological amounts of copper. Although the dendrimers showed potent antioxidant activities against carbon and nitrogen free radicals, EPR and DNA protection studies revealed lack of effectiveness of these dendrimers against hydroxyl radicals. The dendrimers were not cytotoxic to CHO-K1 cells.

List of abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; $CDCl_3$, deuterated chloroform; CHO-K1, Chinese hamster ovary cells; $CuCl_2$, cupric chloride; DMSO, dimethylsulfoxide; DMF, dimethylformamide; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; DMPO-OH, 5,5-dimethyl-1-pyrroline *N*-oxide spin trap and hydroxyl radical adduct; DNA, deoxyribonucleic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ESI-TOF, electrospray ionization-time of flight; ESR, electron spin resonance; ET, electron transfer; $FeSO_4$, ferrous sulfate; HAT, hydrogen atom transfer; H_2O_2, hydrogen peroxide; LDL, low density lipoprotein; $MgSO_4$, magnesium sulfate; MTT, 3-(4,5-di-methylthizol-2-yl)-2,5-diphenyltetrazolium bromide; NaH, sodium hydride; $Na(OAc)_3BH$, sodium triacetoxyborohydride; NaN_3, sodium azide; OC, open circular form of DNA; PAGE, polyacrylamide gel electrophoresis; pBR 322, plasmid DNA; PBS, phosphate-buffered saline; PETN, pentaerythritol; TEA, triethylamine; SC, supercoiled form of DNA; V/V, volume to volume.

* Corresponding author. Tel.: +1 989 774 3289.
** Corresponding author. Tel.: +1 989 774 3303.
E-mail addresses: lee1cy@cmich.edu (C.Y. Lee), sharm1a@cmich.edu (A. Sharma).

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1. Introduction

Free radicals are produced during metabolism and inflammation. These highly reactive species are neutralized by antioxidant enzymes or endogenous/dietary antioxidants. Within cells, antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase play an important role in preventing oxidation of biomolecules. However, in extracellular fluids, key antioxidants include proteins that bind metal ions such as ceruloplasmin, transferrin and albumin and exogenous small molecules such as vitamins C and E and dietary polyphenols [1]. Excess free radicals are often associated with oxidative stress, which has been implicated in various diseases such as cancer, cardiovascular and neurological disorders [2–4]. Antioxidants work in many different ways such as quenching singlet oxygen, binding metal ions, and scavenging free radicals [1]. The majority of antioxidants including polyphenols scavenge free radicals. In this process, the antioxidant transfers hydrogen atom (HAT) or electron (ET) to neutralize the free radical [5,6], thereby preventing chain reactions such as lipid peroxidation. Other antioxidants prevent free radical formation by chelating transition metal ions such as Fe^{2+} and Cu^{+}, which are known to generate free radicals by reaction with hydrogen peroxide [7,8]. Unfortunately, many antioxidants are associated with deleterious pro-oxidant effects during which transition metal ions, such as Cu^{2+} and Fe^{3+} are reduced by the antioxidants to lower oxidation states (Cu^{+} and Fe^{2+}) [9–12]. The reduced metal ions subsequently react with H_2O_2 to form powerful hydroxyl radicals, which are capable of damaging cellular components including proteins, lipids, and nucleic acids.

There are a variety of free radicals with different aqueous solubilities and atomic make-ups that are found in hydrophilic or lipophilic compartments of the human body. Therefore, it is not surprising that a given antioxidant is unable to quench all types of free radicals. A desirable antioxidant should show strong radical scavenging but no pro-oxidant activities. To achieve this goal of designing an ideal antioxidant, we reported synthesis of a new class of antioxidants termed as antioxidant dendrimers [13,14]. The surface of the antioxidant dendrimer is composed of free radical scavenging moieties such as phenolic groups while the core of the dendrimer contains groups that sequester metal ions. Our initial success with antioxidant dendrimers exhibiting potent antioxidant activities and reduced pro-oxidant effects inspired us to synthesize and evaluate other dendritic antioxidants with different cores. The construction of a library of such novel compounds will help us understand their structure–activity relationships. In this study we have synthesized dendrimers with varying amounts of surface electron donating groups (EDG) in order to evaluate the influence of EDG on their antioxidant properties. A pair of dendrimers with polyether cores was prepared from syringaldehyde (two methoxy groups), another pair from vanillin (one methoxy group) and a third pair from 4-hydroxybenzaldehyde (no methoxy group). In the syringaldehyde and vanillin dendrimers, the methoxy groups were *ortho* to the hydroxyl group. Natural and synthetic phenolic compounds with EDG at *ortho* or *para* position, were reported to be better antioxidants than those with *meta*-EDG [15,16]. Synthesis was performed via microwave-assisted 1,3-dipolar cycloaddition utilizing copper metal as a catalyst. The antioxidant ability of these dendrimers to scavenge organic carbon and nitrogen radicals as well as hydroxyl radicals was examined by DPPH assay, DNA and lipoprotein protection studies, and electron spin resonance. Their pro-oxidant effects on copper-mediated DNA damage and cell toxicity were also evaluated.

2. Materials and methods

Syringaldehyde, vanillin, 4-hydroxybenzaldehyde, pentaerythritol (97%), methyl-α-D-glucopyranoside, sodium ascorbate, quercetin, sodium triacetoxyborohydride ($Na(OAc)_3BH$), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Fat Red 7B, phosphate-buffered saline (PBS), potassium persulfate, glacial acetic acid, sodium acetate and methanol were purchased from Sigma Aldrich and were used without further purification. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Cayman Chemical (Ann Arbor, MI, USA). Human low-density lipoprotein (LDL) was obtained from Kalen Biomedical (Montgomery Village, MD, USA). The lipoprotein solution (protein = 5 mg/mL) contained 154 mM NaCl and 0.34 mM EDTA.

^{1}H NMR spectra were recorded with a Varian Mercury spectrometer operating at 500 MHz. ^{13}C NMR spectra were recorded using a Varian Mercury spectrometer operating at 126 MHz.

ESI mass spectra were obtained using a Waters LCT Premier XE mass spectrometer. The source capillary voltage was 3000 V, cone voltage was 10 V and source temperature was 80 °C. Samples analyzed by flow injection had a desolvation gas temperature of 250 °C and a gas flow rate of 200 L/hr. The mobile phase was water (50%)–acetonitrile (50%) containing 0.1% formic acid and its flow rate was 50 μL/min. The injection volume was 10 μL with a sample concentration of approximately 10 ng/uL.

Hitachi HPLC, consisting of L-7200 autosampler, L-7100 pump, L-7400 UV detector and D-7000 interface, was used to analyze the purity of the final dendrimers by reversed phase chromatography. Mobile phase was an acetonitrile-H_2O gradient system (5 → 95% acetonitrile) with 0.1% trifluoroacetic acid. The sample was detected at 214 nm. Flow rate was 1 mL/min. Separation was performed on a Varian C18 RP column.

All spectrophotometric data were obtained using Perkin Elmer UV/Vis spectrometer (Lambda 20) and Molecular Devices Corp. Spectra Max ($M2^{e}$).

The microwave used for the click chemistry was a CEM brand Discover SP v 2.15.

2.1. General procedures for the synthesis of building block

Building block **1a** and **1b** were synthesized as previously described [17].

2.1.1. Building block ***1c***

To synthesize the building block, 250 mL of distilled THF was added to a 500 mL round bottom flask. 4-Hydroxybenzaldehyde (3.56 g, 29.15 mmol) was then dissolved into the THF. Propargylamine (3.027 g, 54.96 mmol) was then added to the solution dropwise via a syringe. The solution was then heated at 40–45 °C. Sodium tri-acetoxyborohydride ($Na(OAc)_3BH$) (6.10 g, 28.78 mmol) was then added to the reaction mixture after the reaction was cooled to room temperature. After 24 h, a second equivalent of 4-hydroxybenzaldehyde (3.56 g, 29.15 mmol) was added to the reaction mixture without heating. After 36 h the last equivalent of reducing agent was added ($Na(OAc)_3BH$) (6.10 g, 28.78 mmol) and the reaction was left to proceed for two days.

The reaction mixture was then filtered under reduced pressure and the filtrate was dried on the rotovap. The resulting residue was re-dissolved in 200 mL chloroform and then washed with 50 mL of water three times. The aqueous layer was extracted with 50 mL of methylene chloride twice. The combined organic layer was dried over magnesium sulfate. After evaporating the organic layer on a rotovap, the residue was purified using flash column chromatography equipped with a 40 g pre-packed silica gel