Archives of Biochemistry and Biophysics 631 (2017) 1–10

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Tetramer as efficient structural mode for organizing antioxidative carboxylic acids: The case in inhibiting DNA oxidation

Ri Zhou, Zai-Qun Liu*

Department of Organic Chemistry, College of Chemistry, Jilin University, Changchun 130021, China

ARTICLE INFO

Article history:
Received 15 June 2017
Received in revised form
27 July 2017
Accepted 3 August 2017
Available online 5 August 2017

Keywords:
DNA oxidation
Tetramer
Antioxidant
Free radical

ABSTRACT

To overcome the problem on the relationship of antioxidative effect with the branch number in a tetramer, we herein designed a series of antioxidants with pentaerythritol, glycerol, and ethylene glycol as the cores, and gallic, ferulic, caffeic, and *p*-hydroxybenzoic acids as the antioxidative moieties. In the case of DNA oxidation mediated by 2,2′-azobis(2-amidinopropane hydrochloride, AAPH), it was found that the *stoichiometric factor* (n) of a carboxylic acid increased rapidly when the acid was esterified with ethylene glycol, glycerol, and pentaerythritol to form a dimer, trimer, and tetramer, respectively. Interestingly, the coefficient in the equation of n~{branch} ({branch} referred to the number of branches) was higher than one, indicating that the antioxidative effect was enhanced more promptly than the increase of the number of branches. Meanwhile, tetramer exhibited high intercalation effect with DNA strand. Therefore, additionally antioxidative effect was ascribed to the tethering effect resulting from tetrameric structure and strong intercalation with DNA strand generated by tetramer.

1. Introduction

Dendrimers were three-dimensional molecules with multiple branches as topological features, which can be tailored and controlled [1]. The stretched branches were beneficial for transferring photoemission energy owing to avoiding the accumulation of conjugation systems [2], while the solvation effect also affected the aggregation states of flexible branches and influenced properties of dendrimers in different surroundings [3]. The spacious skeleton of dendrimers facilitated bioactive molecules across various membranes and barriers by the cellular internalization [4]. A submicrometer-sized nucleodendrimer was produced to transport DNA modules into cells with high specificity [5]. Thus, the well-defined molecular architecture and tunable chemical composition allowed dendrimers to be powerful carriers for therapeutic and diagnostic agents [6] and drugs [7]. In this case, endeavors have been contributed to attach the branch block to an appropriate core *via* convenient operations [8]. However, the blossom in the study on dendrimers still did not reveal whether the superiority of dendrimers was achieved by increasing the number and the length of branches. It was necessary to clarify the relationship between the dendrimer property and the branch number.

The usage of antioxidants to scavenge reactive oxygen species (ROS) has emerged as a therapeutic pathway for oxidative stress-related diseases [9], during which dendrimer was found to be a valid mode for organizing antioxidative molecules devoid of prooxidative drawbacks [10]. We herein aimed at exploring the correlation of the antioxidative effects with the number of branches. Although much of the current perspective was focused on the application of commercial dendrimers with a large number of branches bearing complicated functionalized groups [11], simply natural polyphenols such as gallic acid [12], ferulic acid [13], and caffeic acid [14] still played the key role in the design of polymeric antioxidants. To observe the variation on antioxidant effects from dimer to trimer and tetramer was capable of exploring the structure-activity relationship. Over the past decade, cores applied for constructing dendrimers were developed from simple molecules [15] to porphyrin [16], metallic cluster [17], and carbon nanotube [18], *etc*. Of these metrics, as depicted in Fig. 1, some small organic molecules such as pentaerythritol [19], glycerol [20], and ethylene glycol [21] were able to bear four, three, and two branches for producing tetramer, trimer, and dimer, respectively.

Among a large number of biological species undergoing oxidation, the oxidative damage of DNA was regarded to be the reason for a number of pathological disorders [22]. Numerous reactive sites in

* Corresponding author.
E-mail address: zaiqun-liu@jlu.edu.cn (Z.-Q. Liu).

http://dx.doi.org/10.1016/j.abb.2017.08.002
0003-9861/

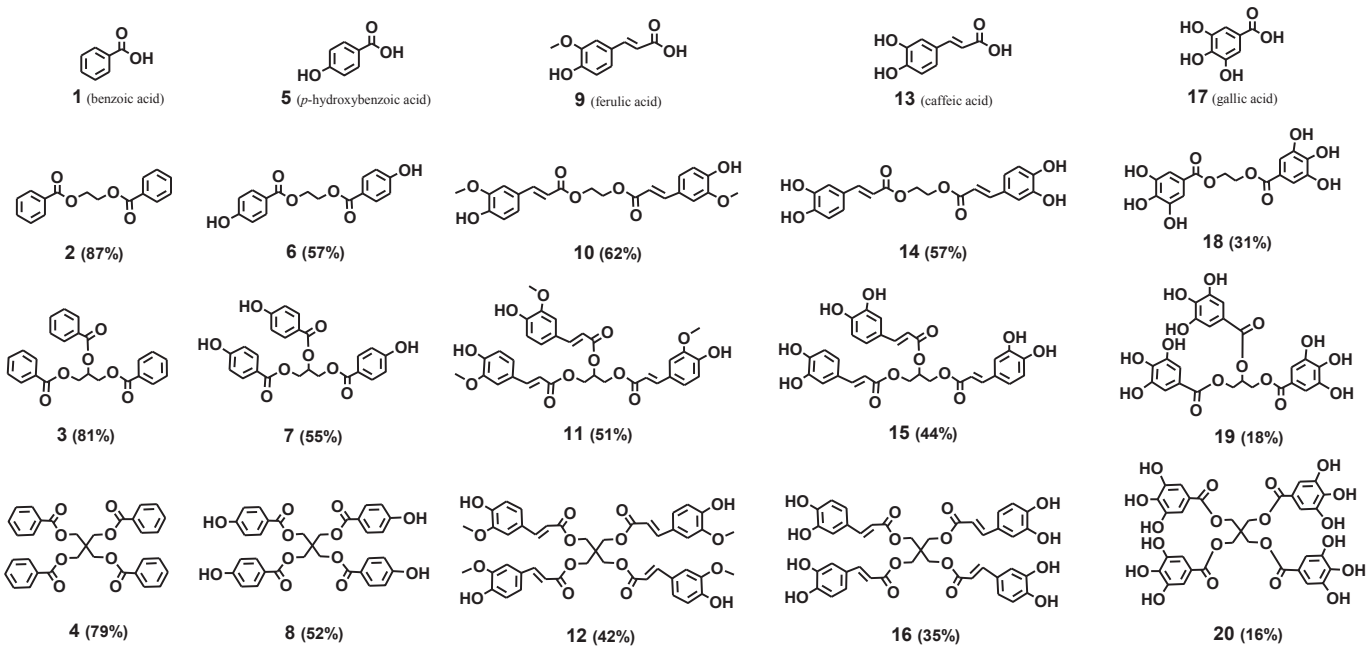

Fig. 1. Antioxidants employed as dimers, trimers, and tetramers.

DNA were susceptible to be attacked by •OH, Cu^{2+}/glutathione (GSH), and peroxyl radical [23]. We herein compared inhibitory effects of compounds (as shown in Fig. 1) on DNA oxidations mediated by •OH, Cu^{2+}/GSH, and peroxyl radical [24] in order to reveal the correlation of antioxidative effects with the number of branches.

2. Experimental section

2.1. Materials and instrumentation

2,2′-Azobis(2-amidinopropane hydrochloride) (AAPH) and naked DNA sodium salt were products of ACROS Organics, Geel, Belgium, and used as received. Solvents and reagents used in the synthesis were obtained commercially and used as such unless noted otherwise. All of the products were identified by ^1H and ^{13}C NMR spectroscopy (Bruker Avance III 400 MHz spectrometer) as shown in the Supporting Information, and the molecular weight was detected by high resolution mass spectra (HRMS) equipped with ESI as the ionization mode (Agilent 1290-micrOTOF Q II).

2.2. Synthesis of tetramers, trimers, and dimers

Briefly, to 20 mL of $CHCl_3$ was added 1.2 mL of benzoyl chloride (10.5 mmol), followed by adding 0.34 g of pentaerythritol (2.5 mmol) at room temperature under nitrogen. Then, 1.5 mL of $(C_2H_5)_3N$ (10.5 mmol) was added dropwise and stirred for 2 h. The reaction was heated at 60 °C and stirred for 3 h. After completion of the reaction as indicated by TLC, 15 mL of water was added to quench the reaction. The aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic layers were dried over Na_2SO_4. After the organic solvent was evaporated, the residue was purified by silica gel chromatograph with petroleum ether/ethyl acetate as eluent to afford 1.09 g of **4** (white crystal, $R_f = 0.44$ in petroleum ether/ethyl acetate = 5/1, *v*/*v*), yield 79%. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.03 (s, 8H), 7.58 (s, 4H), 7.43 (s, 8H), 4.74 (s, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.2, 166.1, 133.4, 129.7, 129.4, 128.5, 63.7, 43.1. HRMS (ESI): calcd for $[M + H^+]$ of $C_{33}H_{28}O_8$: 553.1784, found: 553.1869.

The same operation was carried out as the aforementioned procedure except that pentaerythritol was replaced by glycerol (3.3 mmol) to afford 818 mg of **3** (yellow crystal, $R_f = 0.51$ in petroleum ether/ethyl acetate = 5/1, *v*/*v*), yield 81%. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.09 (d, $J = 4$ Hz, 2H), 8.06 (d, $J = 4$ Hz, 4H), 7.60 (t, $J = 4.0$ Hz, 2H), 7.58 (t, $J = 4.0$ Hz, 1H), 7.47 (t, $J = 4.0$ Hz, 4H), 7.44 (t, $J = 4.0$ Hz, 2H), 5.87 (m, 1H), 4.75 (ddd, $J = 17.8, 11.9, 5.1$ Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.1, 165.7, 133.4, 133.2, 129.8, 129.7, 129.5, 128.5, 69.8, 63.0. HRMS (ESI): calcd for $[M + H^+]$ of $C_{24}H_{20}O_6$: 405.1260, found: 405.1304. And 587 mg of **2** (white crystal, $R_f = 0.61$ in petroleum ether/ethyl acetate = 5/1, *v*/*v*) was also obtained by using ethylene glycol (5.0 mmol), yield, 87%. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.09 (d, $J = 7.7$ Hz, 4H), 7.59 (s, 2H), 7.47 (s, 4H), 4.70 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.4, 133.2, 129.9, 129.7, 128.4, 62.8. HRMS (ESI): calcd for $[M + H^+]$ of $C_{16}H_{14}O_4$: 271.0892, found: 271.0913.

The hydroxyl groups in carboxylic acids were protected by acetic anhydride before reacting with $SOCl_2$ to afford acyl chloride. The phenolic hydroxyl group-protected carboxylic acid and $SOCl_2$ (mol ratio, 1:3) were dissolved in $CHCl_3$ under nitrogen atmosphere, and three drops of *N*,*N*-dimethyl formamide (DMF) was added and heated at 60 °C for 3 h under stirring. Then, the organic solvents were removed under vacuum, and the crude acyl chloride was used without further purification. The acyl chloride (10.5 mmol) and pentaerythritol (2.5 mmol), glycerol (3.3 mmol), or ethylene glycol (5.0 mmol) were dissolved in $CHCl_3$ under nitrogen atmosphere, and $(C_2H_5)_3N$ (10.5 mmol) was added and heated at 60 °C for 24 h under stirring. After completion of the reaction as indicated by TLC, water was added to quench the reaction. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic layers were dried over Na_2SO_4. After the organic solvent was removed under vacuum, the residue was purified by silica gel chromatograph with $CH_2Cl_2/CH_3COOC_2H_5$ as the eluent to afford esters, in which phenolic hydroxyl groups were protected by acetyl groups.

The acetyl groups in the obtained esters were removed by CH_3COONH_4. Briefly, an ester was dissolved in $CHCl_3$, followed by adding three equivalent of CH_3COONH_4 (dissolved in CH_3OH) and