Communication

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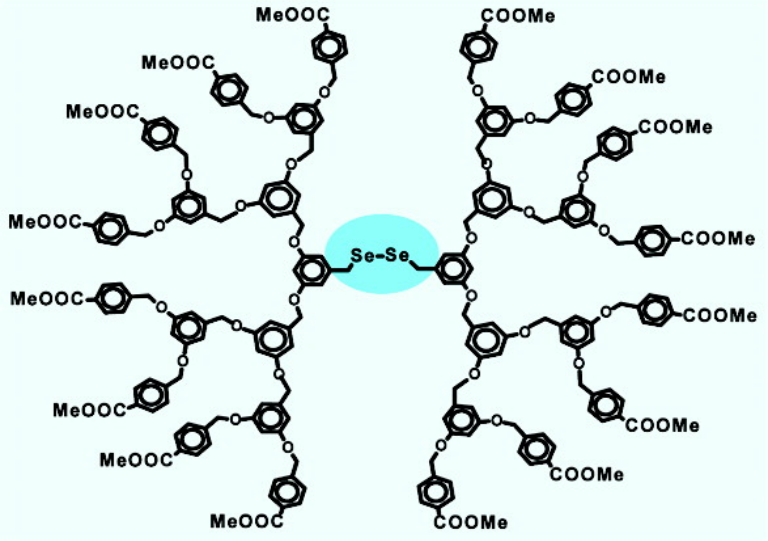

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Highly Efficient Dendrimer-Based Mimic of Glutathione Peroxidase

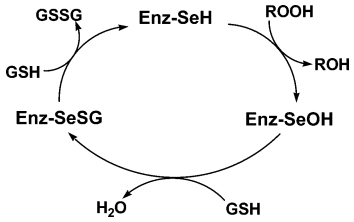
Xi Zhang,*,† Huaping Xu,†,‡ Zeyuan Dong,‡ Yapei Wang,† Junqiu Liu,*,‡ and Jiacong Shen‡

Key Lab of Organic Optoelectronics & Molecular Engineering, Department of Chemistry, Tsinghua University, Beijing 100084, P. R. China, and Key Lab for Supramolecular Structure & Materials, College of Chemistry, Jilin University, Changchun 130023, P. R. China

Received February 27, 2004; E-mail: xi@mail.tsinghua.edu.cn; jqliu2000@yahoo.com

In this communication, we report the synthesis of the three generations of Fréchet-type poly(aryl ether) dendrimers with a diselenide core that demonstrate generation-dependent glutathione peroxidase (GPx) activity with initial reduction rates as high as 2431.20 μM min^{-1} for the third-generation product. GPx is a mammalian antioxidant seleno-enzyme that protects biomembranes and other cellular components from oxidative damage by catalyzing the reduction of a variety of hydroperoxides (ROOH), using glutathione (GSH) as the reducing substrate (Scheme 1).[1] The catalytic active center of GPx, selenocysteine, is in a depression on the protein surface, in which some charged and hydrophobic amino acid residues (Phe, Trp, Asp) form a hydrophobic cavity.[2] The discovery of Ebselen (2-phenyl-1,2-benzoisoselenazol-3(*2H*)-one), which functions as an antioxidant,[3] has inspired a worldwide interest in the design of GPx mimics.[4] A highly efficient GPx mimic, however, remains a great challenge. For achieving the goal, one of the strategies is to consider the substrate binding and mimic the catalytic microenvironment.[5]

Scheme 1. Catalytic Cycle for GPx



Like natural enzymes, the structures of dendrimers can be precisely controlled at the molecule level, resulting in a well-defined microenvironment.[6] For example, Fréchet et al. used the poly(aryl ether) dendrimer, with an alkoxide group at the core to initiate anionic ring-opening polymerization.[7] Meijer and co-workers demonstrated that the fifth-generation poly(propyleneimine) dendrimer could be used as a "dendritic box" capable of retaining substrates trapped during synthesis and preventing them from diffusing outward.[8] Bruner designed dendritic catalysts containing dendritic phosphines, which he wishfully called "dendrizymes".[9] Detty's group reported the use of dendritic polyphenylchalcogenide to catalyze the oxidation of bromide with hydrogen peroxide.[10] Diederich and co-workers showed that a kind of dendrimer, called a dendrophane, was able to bind arenes and steroids within its macrocyclic core for catalyzing the oxidation of aromatic aldehydes into aromatic esters.[11]

Herein we attempt to take advantage of the microenvironment provided by the delicate structure of the dendrimers and introduce the catalytic center into the inner core of the dendrimer in order to achieve high GPx activity. For this purpose, we have designed and synthesized a series of Fréchet-type poly(aryl ether) dendrimers with a diselenide core. Dendron bromides (CO_2Me)Gn−Br were synthesized according to previous literature.[12] The reaction of NaSeSeNa with (CO_2Me)Gn−Br and the following purification by silica gel column chromatography yielded a yellow powder, the dendrimer with the diselenide core ((CO_2Me)Gn−Se−)$_2$, as shown in Scheme 2. The dendrimers with different generations (Gn, n = 1, 2, 3) were well characterized with ^{1}H NMR, ^{13}C NMR, MALDI-MS, and elemental analysis (see details in Supporting Information).

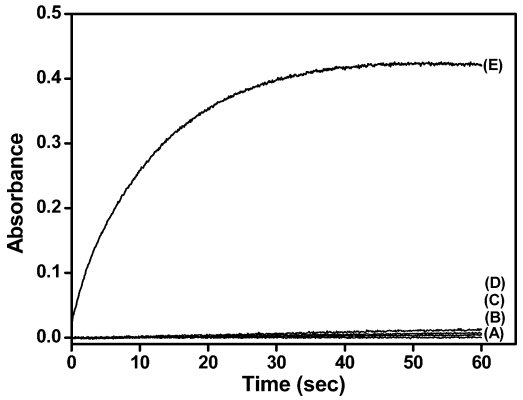


Figure 1. Plots of absorbance vs time during the catalytic reduction of H_2O_2 (2 mM) by PhSH (1 mM) in a solvent mixture of 3:7 chloroform/methanol. Catalysts: (A) none; (B) [Ebselen] = 10.0 μM; (C) [G1] = 38.5 μM; (D) [G2] = 19.4 μM; (E) [G3] = 10.0 μM.

Scheme 2. General Synthetic Route of ((CO_2Me)Gn−Se−)$_2$

$$(CO_2Me)Gn\text{-}Br \xrightarrow{NaSeSeNa} ((CO_2Me)Gn\text{-}Se\text{-})_2$$

We have measured the catalytic activity of the synthesized dendrimer mimics of GPx according to the method reported by Tomoda et al. using benzenethiol (PhSH) as a glutathione alternative.[4i] The initial rates (v_0) for the reduction of H_2O_2 by PhSH in the presence of dendrimer mimics of GPx were determined in a solvent mixture by monitoring the UV absorption of diphenyl disulfide (PhSSPh) at 305 nm (Figure 1). The relative activities of the compounds are summarized in Table 1. For the same solvent mixture of chloroform/methanol (3:7, volume ratio), dendrimers G1 and G2 show relatively low GPx activity, and their initial rates are 4.07 and 8.19 μM min^{-1} respectively. However, for dendrimer G3, we have found interestingly that the initial rate can be as high as 2431.20 μM min^{-1}. To the best of our knowledge, this rate is rather high among the organic systems mimicking GPx. In contrast

† Tsinghua University.
‡ Jilin University.

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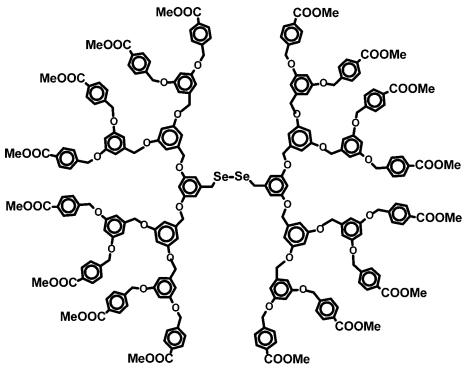
Table 1. Initial Reduction Rates (v_0)[a] of H_2O_2 (2 mM) with PhSH (1 mM) in the Presence of Various Dendrimer Catalysts (0.01 mM) in Different Solvent Mixtures (Volume Ratio)

	v_0 (μM min^{-1})		
catalyst	$CHCl_3/CH_3OH$ 3:7	$CHCl_3/CH_3OH$ 7:3	$CHCl_3/CH_3OH/H_2O$ 3:6.5:0.5
none	0.97 (0.06)	0.71 (0.07)	1.15 (0.13)
G1	4.07 (0.65)	3.29 (0.47)	3.91 (0.86)
G2	8.19 (1.03)	3.61 (0.53)	158.39 (27.89)
G3	2431.20 (46.50)	5.39 (0.68)	2966.48 (167.71)

[a] Obtained by Lineweaver−Burk plots, and standard deviations are shown in parentheses.

to Ebselen catalysis determined under the same conditions, for example, a remarkable rate enhancement of 1400-fold is observed for dendrimer G3.



G3

We wondered whether the high GPx activity of G3 originates from the hydrophobic microenvironment provided by its macromolecular structure. To confirm this speculation, we first measured the binding constants between the dendrimers and PhSH. According to the Hildebrand−Benesi equation, the linear plot of the reciprocal of the absorbance difference and molar concentration of the guest molecule indicates a 1:1 complex between the host molecules and the guest molecule PhSH.[13] The binding constants of dendrimers G1, G2, and G3 are 16.4, 39.4, and 252.7 M^{-1}, respectively, with a slight increase from the first to the second generation but a dramatic increase from the second to the third generation, indicating that dendrimer G3 contains a favorable microenvironment for the catalytic cycle. This speculation can be further supported by computer simulation that the catalytic site is well encapsulated for high-generation G3, forming a hydrophobic cavity.

The microenvironment of dendrimers can be also adjusted by solvent mixture, so the activity should change as the solvent mixture changes. As seen from Table 1, taking G3 as an example, its GPx activity is 2431.20 μM min^{-1} in the solvent mixture chloroform/methanol (3:7); however, it decreases remarkably to 5.39 μM min^{-1} when the solvent mixture is changed into chloroform/methanol (7:3). Considering that the chloroform is a good solvent for G3 whereas methanol is a poor solvent, we expect that in the case of the higher polarity of the solvent mixture, G3 should adopt a more globular conformation that is more favorable for the catalytic cycle. Moreover, when 5% water was added to the solvent mixture, the activity of G3 reached as high as 2966.48 μM min^{-1}. The same trend is also true for G2: the activity is just 8.19 μM min^{-1} in the case of solvent mixture chloroform/methanol (3:7) but increases to 158.39 μM min^{-1} when a little water was added (see Table 1). The enhanced GPx activity after adding water may indicate that hydrophobicity plays a role in the catalytic reaction.

In conclusion, we have prepared a dendrimer-based mimic of GPx that makes use of the microenvironment governed by the molecular architecture as well as by solvent mixture. It represents a successful example of using a dendrimer as a model for a highly efficient GPx mimic. We are modifying the structures of the dendrimers in order to develop several model compounds with different solubilities and catalytic activities. It is anticipated that this work opens a new avenue for high-efficiency mimics of GPx.

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Supporting Information Available: Synthesis and full characterization of the three generations of dendrimers, experimental details for measurements of the GPx catalytic activity and binding constants of the dendritic catalysts, and computer simulation of G3 conformation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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