Hyperbranched polyselenides as glutathione peroxidase mimics†

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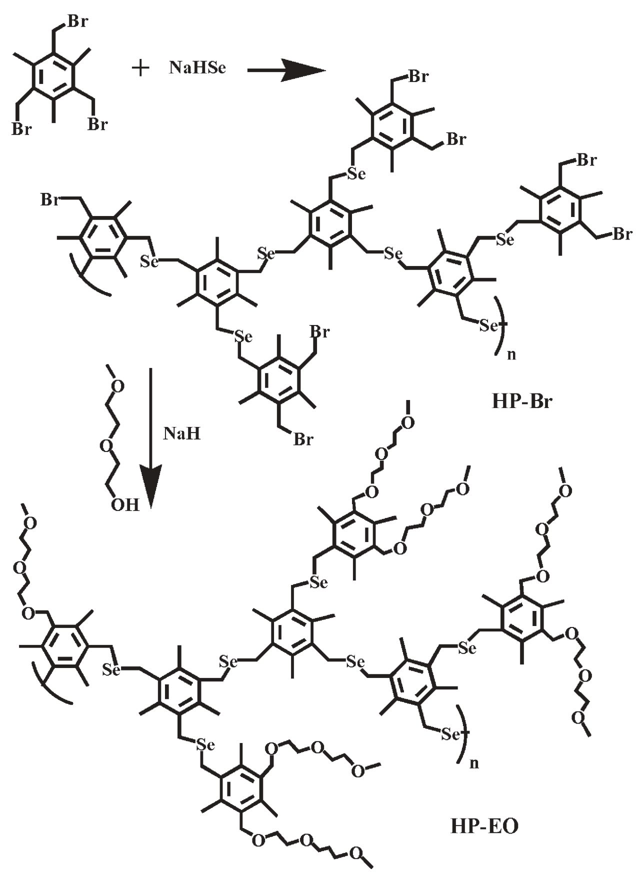
Novel hyperbranched polyselenides with multi-catalytic sites at the branching units have been synthesized which may provide a new approach towards glutathione peroxidase mimics.

In this communication we report the synthesis of hyperbranched polyselenides with multi-catalytic sites of selenium located at the branching units, which provides a novel model for glutathione peroxidase (GPx) mimics. GPx is a mammalian antioxidant enzyme that protects biomembranes and other cellular components from oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione (GSH) as the reducing substrate. The catalytically active center of GPx is selenocysteine.[1] The discovery of Ebselen (2-phenyl-1,2-benzoisoselenazol-3(2*H*)-one), which functions as an antioxidant, has inspired a worldwide interest in the design of GPx mimics.[2,3]

Recently by taking advantage of the microenviroment provided by the dendrimers and introducing catalytic groups (diselenide) into the core of the dendrimers we have successfully achieved high GPx activity.[4] Like dendrimers, hyperbranched polymers (HBPs) have attracted increasing scientific and industrial attention in recent years due to their unusual chemical and physical properties such as compact three-dimensional structures, large numbers of terminal functional groups, and low intrinsic viscosities.[5] Their applications vary mainly from coatings and additives to light emitting materials and so on.[5,6] However the use of HBPs as models for biomimetics or as catalytically active molecules is still in its infancy.[7] The normal way to use HBPs as catalysts is to functionalize the peripheries with catalytic groups. Few efforts have been tried to introduce catalytic groups at the inner branching units. Herein we have succeeded in introducing selenium in the branching units of HBPs, which act as GPx mimics.

In many cases, hyperbranched polymers are synthesized by self-polycondensation of AB*n* type monomers which have one "A" and *n* "B" functional groups. However, in our cases, the polycondensation of A_2 (or AA′) monomers with B_3 monomers seemed more attractive as the required starting materials can be obtained more readily.[8] Herein we used a direct polymerization of NaHSe (as the AA′ monomer) and 1,3,5-tris-bromomethyl-2,4,6-trimethyl-benzene (as the B_3 monomer) in a ratio of 1 : 1 leading to new hyperbranched polyselenides as shown in Scheme 1. For synthetic details see ESI.† As can be seen from the ^{1}H-NMR spectrum of HP-Br in Fig. 1 A, a new broad peak appears at around 3.80 ppm which can be ascribed to the Ar–CH_2–Se– unit after polymerization. A single peak appears around 196.3 ppm in the ^{77}Se-NMR spectrum, shown in Fig. 1 B, which indicates that selenium is indeed interposed into the HBP exclusively as –CH_2–*Se*–CH_2–, instead of in other oxidized states. NaHSe was completely consumed during the reaction, since it was very reactive. Thus the ratio of Se to Br in the resulting polymer should be 1 : 1, which was supported by XPS data (ratio of Se : Br was 1.05 : 1). The molecular weight was measured by conventional gel permeation chromatography (GPC) performed in CH_2Cl_2 against linear polystyrene standards. The apparent M_w was around 1780 g mol^{-1} with a polydispersity of 1.08. It should be emphasized that the molecular weights obtained by GPC are likely to be underestimated because of the compact structure of our



Scheme 1 Synthetic route to hyperbranched polyselenides HP-Br and HP-EO.

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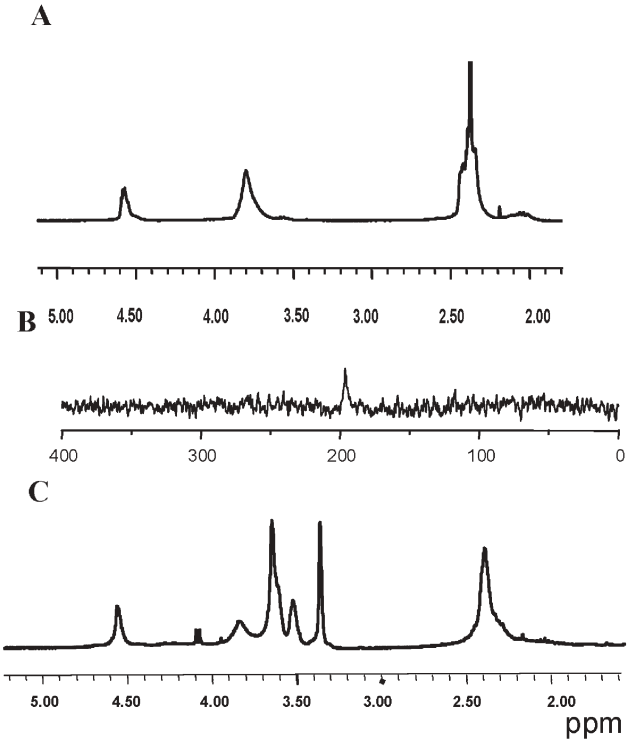

Fig. 1 ^{1}H-NMR and ^{77}Se-NMR of HP-Br and HP-EO: (A) ^{1}H-NMR of HP-Br; (B) ^{77}Se-NMR of HP-Br; (C) ^{1}H-NMR of HP-EO.

macromolecule.[9] The above characterization confirms the successful synthesis of HP-Br.

We have measured the catalytic activity of the synthesized hyperbranched polyselenides HP-Br in organic solvents according to the method reported by Tomoda *et al.* using benzenethiol (PhSH) as a glutathione alternative.[3f] The initial rates (v_0) for the reduction of H_2O_2 by PhSH in the presence of hyperbranched polyselenides were determined in a solvent mixture of 1 : 9 chloroform–methanol by monitoring the UV absorption of diphenyl disulfide (PhSSPh) at 305 nm (Fig. 2). The initial reduction rate of H_2O_2 without any catalyst was around 0.38 μM min^{-1}. After the addition of hyperbranched polyselenides with a concentration of 1.32 mg per 100 ml, the initial reduction rate increased rapidly to 6.34 μM min^{-1}. In order to determine whether the reactivity of the hyperbranched polyselenides on a molar basis is a function of the total number of individual reactive selenium groups as well as the reactivity of the individual groups or not, we synthesized an organic monoselenide BDB-Se (bis(3,5-dimethylbenzyl) selenide, see ESI†) for comparison. The initial reduction rate in the presence of BDB-Se was around 1.72 μM min^{-1}. The difference in the reduction rate between HP-Br and BDB-Se can be clearly seen from Fig. 2. The initial reduction rate of the hyperbranched polyselenides is around 3.7 times that of the comparative organic monoselenide BDB-Se. The theoretical selenium weight percentage in both compounds is almost the same, 24.82% for the hyperbranched polyselenides and 24.88% for BDB-Se respectively. So obviously the incorporation of numerous catalytic groups in the macromolecule is important for the higher activity of the hyperbranched polyselenides. The local concentration of selenium in the hyperbranched polymer is very high. We think the advantage of hyperbranched polyselenides

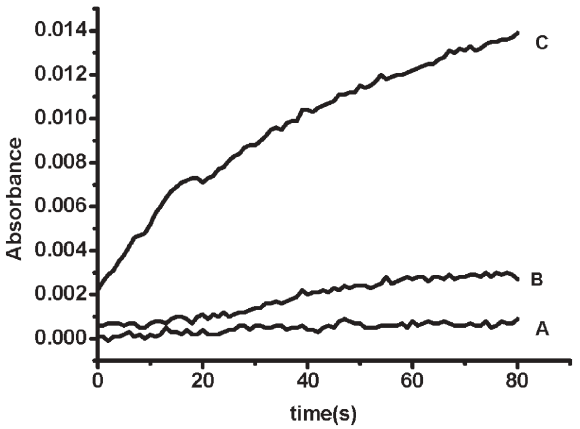

Fig. 2 Plots of the absorbance at 305 nm *vs.* time during the catalytic reduction of H_2O_2 (0.25 mM) by PhSH (1 mM) in a solvent mixture of 1 : 9 chloroform–methanol. Catalysts: (A) none, v_0 = 0.38 μM min^{-1}; (B) BDB-Se 1.32 mg per 100 ml, v_0 = 1.72 μM min^{-1}; (C)HP-Br 1.32 mg per 100 ml, v_0 = 6.34 μM min^{-1}.

during the catalysis may result from an increased chance for the substrate to get close enough to an active catalytic site.

Water solubility of organic compounds is frequently required for evaluation in biological systems and for real applications in this context. Hence, the periphery of HP-Br was successfully functionalized with oligo-ethylene oxide groups in the presence of NaH as shown in Scheme 1. In this way the hyperbranched polyselenide HP-EO was obtained, endowed with enhanced hydrophilicity and water solubility. From the ^{1}H-NMR spectrum (Fig. 1 C) we can easily see that oligo-ethylene oxide chains have been attached to the hyperbranched polyselenides. This was confirmed by GPC measurements in THF showing an increase of the apparent molar mass to M_w = 2761 with a polydispersity of 1.28. The activity of the peripherally EO substituted hyperbranched polyselenides in aqueous solution was assessed using GSH as a thiol substrate in the classical coupled reductase assay system under identical experimental conditions. The oxidation of GSH to the corresponding disulfide (GSSG) was measured indirectly by the spectrophotometric monitoring of the oxidation of NADPH in the presence of glutathione reductase at 340 nm.[3e] The EO functionlized hyperbranched polyselenides showed a GPx activity of 0.28 U mg^{-1}. This relatively low value can be explained on the basis of steric hindrance from the big EO chains, lowering the chance of the substrate to penetrate to the catalytic sites. However, it seems reasonable that there remains a great potential for us to increase the GPx activity by fine-tuning the peripheral structures of the hyperbranched polyselenides.

In conclusion, we have successfully synthesized a novel hyperbranched polyselenide with multi-catalytic sites at the branching units which acts as a novel glutathione peroxidase mimic. To the best of our knowledge, this may be the first successful example of incorporating catalytic sites onto the skeleton of the hyperbranched polymer. By post-synthetic modification of the periphery this hyperbranched polyselenide could be solubilized in water, which may be very important for real use as an antioxidant drug. It is believed that this work will open a new avenue for biocatalysis by hyperbranched polymers.

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Notes and references

1 (*a*) F. Ursini, M. Maiorino, R. Brigelius-Flohé, K. D. Aumann, A. Roveri, D. Schomburg and L. Flohé, *Methods Enzymol.*, 1995, **252**, 38; (*b*) O. Epp, R. Ladenstein and A. Wendel, *Eur. J. Biochem.*, 1983, **133**, 51.

2 A. Müller, E. Cadenas, P. Graf and H. Sies, *Biochem. Pharmacol.*, 1984, **33**, 3235.

3 (*a*) G. Mugesh and H. B. Singh, *Chem. Soc. Rev.*, 2000, **29**, 347; (*b*) G. Mugesh, W.-W. du Mont and H. Sies, *Chem. Rev.*, 2001, **101**, 2125; (*c*) H. Sies, *Free Radical Biol. Med.*, 1993, **14**, 313; (*d*) T. G. Back and B. P. Dyck, *J. Am. Chem. Soc.*, 1997, **119**, 2079; (*e*) S. R. Wilson, P. A. Zucker, R.-R. C. Huang and A. Spector, *J. Am. Chem. Soc.*, 1989, **111**, 5936; (*f*) M. Iwaoka and S. Tomoda, *J. Am. Chem. Soc.*, 1994, **116**, 2557; (*g*) G. Mugesh, A. Panda, H. B. Singh, N. S. Punekar and R. J. Butcher, *J. Am. Chem. Soc.*, 2001, **123**, 839; (*h*) L. Engman, D. Stern, I. A. Cotgreave and C. M. Andersson, *J. Am. Chem. Soc.*, 1992, **114**, 9737; (*i*) L. Engman, D. Stern, M. Pelcman and C. M. Andersson, *J. Org. Chem.*, 1994, **59**, 1973; (*j*) M. R. Detty, A. E. Friedman and A. R. Oseroff, *J. Org. Chem.*, 1994, **59**, 8245; (*k*) G. M. Luo, X. J. Ren, J. Q. Liu and J. C. Shen, *Curr. Med. Chem.*, 2003, **10**, 1151; (*l*) Z. Y. Dong, J. Q. Liu, S. Z. Mao, X. Huang, B. Yang, X. J. Ren, G. M. Luo and J. C. Shen, *J. Am. Chem. Soc.*, 2004, **126**, 16395.

4 X. Zhang, H. P. Xu, Z. Y. Dong, Y. P. Wang, J. Q. Liu and J. C. Shen, *J. Am. Chem. Soc.*, 2004, **126**, 10556.

5 (*a*) Y. H. Kim, *J. Polym. Sci., Part A: Polym. Chem.*, 1998, **36**, 1685; (*b*) B. Voit, *J. Polym. Sci., Part A: Polym. Chem.*, 2000, **38**, 2505; (*c*) M. Jikei and M. Kakimoto, *Prog. Polym. Sci.*, 2001, **26**, 1233; (*d*) C. Gao and D. Yan, *Prog. Polym. Sci.*, 2004, **29**, 183.

6 (*a*) J. Chen, H. Peng, C. C. W. Law, Y. Deng, J. W. Y. Lam, I. D. Williams and B. Tang, *Macromolecules*, 2003, **36**, 4319; (*b*) J. Li and Z. Bo, *Macromolecules*, 2004, **37**, 2013; (*c*) M. Smet, E. Schacht and W. Dehaen, *Angew. Chem., Int. Ed.*, 2002, **41**, 4547.

7 (*a*) C. Gao, Y. Xu, D. Yan and W. Chen, *Biomacromolecules*, 2003, **4**, 704; (*b*) P. Kolhe, J. Khandare, O. Pillai, S. Kannan, M. Lieh-Lai and R. Kannan, *Pharm. Res.*, 2004, **21**, 2185.

8 (*a*) T. Emrick, H. Chang and J. M. J. Fréchet, *Macromolecules*, 1999, **32**, 6380; (*b*) M. Jikei, S. Chon, M. Kakimoto, S. Kawauchi, T. Imase and J. Watanebe, *Macromolecules*, 1999, **32**, 2061.

9 Y. Lim, S. M. Kim, Y. Lee, W. Lee, T. Yang, M. Lee, H. Suh and J. Park, *J. Am. Chem. Soc.*, 2001, **123**, 2460.

