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ORIGINAL ARTICLE

Evaluating substrate specificity of glutathione peroxidase mimic by molecular dynamics simulations and kinetics

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Abstract The substrate specificities of glutathione peroxidase (GPX) mimic, 6,6′-ditellurobis(6-deoxy-β-cyclodextrin) (6-TeCD), for three hydroperoxides (ROOH), H_2O_2, *tert*-butyl hydroperoxide (*t*-BuOOH) and cumene hydroperoxide (CuOOH), are investigated through molecular dynamics (MD) simulations. The most stable conformations and the total interaction energies of complex of 6-TeCD with ROOH are used to evaluate the substrate specificity of 6-TeCD. The steady-state kinetics of 6-TeCD is studied and the Michaelis-Menten constant (K_m) and second-order rate constant k_{max}/K_{ROOH} show that 6-TeCD displays different affinity and specificity to ROOH. These results of experiments are well consistent with ones obtained by MD simulations, indicating that MD simulations could be applied to evaluation substrate specificity of small-molecule enzyme mimics.

Keywords Glutathione peroxidase · Enzyme mimic · Substrate specificity · Molecular dynamics simulations · Kinetics

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Abbreviations

GPX	Glutathione peroxidase
GSH	Glutathione
t-BuOOH	*Tert*-butyl hydroperoxide
CuOOH	Cumenyl hydroperoxide
Ebselen	2-Phenyl-1,2-benzoisoselenazol-3(*2H*)-one
NADPH	β-Nicotinamide adenine dinucleotide phosphate
β-CD	β-Cyclodextrin
PBS	Potassium phosphate buffer
MD	Molecular dynamics

Introduction

Glutathione peroxidase (GPX, EC 1.11.1.9) is a well-known selenoenzyme which catalyzes the reduction of harmful ROOH by glutathione (GSH) and protects the lipid membranes and other cellular components against oxidative damage [1–4]. Because the natural GPX has some shortcomings (instability, antigenicity and poor availability), scientists have paid more attention to its artificial imitation [5, 6]. 2-phenyl-1, 2-benzoisoselenazol-3(*2H*)-one (Ebselen) is a well known GPX mimic [5]. In recent years, more synthetic selenium/tellurium compounds have been made as mimics of GPX, not only for elucidating catalytic mechanism, but also for potential medicinal application [7, 8]. But, most of artificial GPX mimics have low GPX activity because they are short of substrate binding site.

β-Cyclodextrin (β-CD) molecule has a hydrophilic outer surface and a hydrophobic inner core, conducive to the formation of inclusion complexes through binding of various hydrophobic compounds and small molecules into

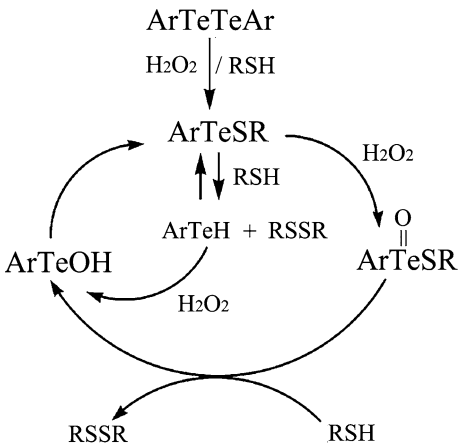
their hydrophobic cavities [9]. The interactions of β-CD and guest molecules are directed, specific and reversible, and a wealth of information is available concerning their binding strength and kinetics [9, 10]. Consequently, β-CD molecule as a variety of enzyme models are useful implements for studying the natural enzymes [11, 12]. Recently, we have reported a new β-CD-based GPX mimic, 6,6′-ditellurobis(6-deoxy-β-cyclodextrin) (6-TeCD), with different substrate specificity for ROOH [13].

Using a ^{1}H NMR method, Engman and co-worker have studied the reaction kinetics of diaryl ditelluride (GPX mimic) and its reaction mechanism is shown in Scheme 1 [14]. We have recently reported mechanism of 2-TeCD, which catalyze the reduction of ROOH by 3-carboxy-4-nitrobenzenethiol (ArSH), as shown in Scheme 2 [15]. These works suggest that the bridge ditellurides ruptured in the catalytic cycle and recognition by β-CD is primary effect to substrate binding. Therefore, we investigate the substrate specificity and catalytic efficiency of 6-TeCD for different ROOH by means of MD simulations and its catalytic kinetics.

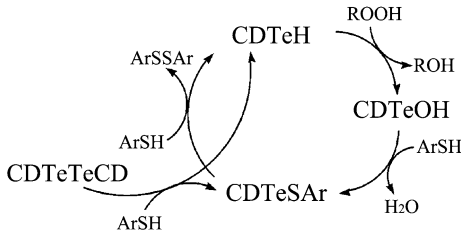
Experimental and computational details

Apparatus and materials

The spectrometric measurements were carried out using a Shimadzu UV-2550 spectrophotometer. β-CD was purchased from Shanghai Sanpu Chemical Plant. 6-TeCD was synthesized and purified according to the literature [13], GSH, glutathione reductase, *t*-BuOOH, CuOOH, Ebselen and β-nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma-Aldrich. All the other materials were of analytical grade and used without further purification.



Scheme 1 Proposed mechanism of the thiol peroxidase reaction of ditellurides



Scheme 2 Proposed Catalytic Mechanism of 2-TeCD

MD simulations and docking study

All simulations were performed on SGI O38600 workstations using the Insight II software package developed by Biosym Technologies [16]. The consistent-valence forcefield (CVFF) was used for energy minimization (EM) and MD simulations. The initial three-dimensional (3D) model of β-CD was taken from three-dimensional crystal structures [17] and improved by EM. After performing 200 steps of conjugate gradient (CG) minimization, an MD simulation was carried out to examine the quality of the model structures by checking their stability via 100 ps simulations at 310 K (37 °C). An explicit solvent model TIP3P water was used [18]. All of the 3D models were solvated in a sphere of TIP3P water molecules with radius 20 Å, and their protonation states were set at pH 7.0. Finally, a CG EM of the full complexes was performed until the root mean-square (RMS) gradient energy was lower than 0.001 kcal mol^{-1}. All of the calculations mentioned above were done with the Discover-3 software package [19].

Affinity, which uses a combination of Monte Carlo type and Simulated Annealing procedure to dock, is a suite of programs for automatically docking a ligand (guest) to a receptor (host) [20]. By means of the 3D structures of ROOH, which are built through the Insight II/Builder program, the automated molecular docking is performed by using docking program affinity. A key feature is that the "bulk" of the receptor, defined as atoms which are not in the binding (active) site specified, is held rigid during the docking process, while the binding site atoms and ligand atoms are movable. The potential function of the complex is assigned by using the CVFF and the cell multipole approach is used for nonbonding interactions. To account the solvent effect, the centered β-CD-ROOH complexes are solvated in a sphere of TIP3P water molecules with radius 20 Å. Finally, the docked complexes of β-CD with H_2O_2, *t*-BuOOH or CuOOH, are selected by the criteria of interacting energy combined with the geometrical matching quality. These complexes are used as the starting

conformation for further EM and geometrical optimization before the final models are achieved.

Assay of GPX kinetics

The assay of 6-TeCD kinetics is similar to that for native GPX [21]. The initial rates for reduction of H_2O_2, *t*-BuOOH and CuOOH by GSH are determined by observing the change of NADPH absorption at 340 nm (Eqs. 1 and 2)

$$\mathrm{ROOH} + 2\mathrm{GSH} \xrightarrow{\mathrm{GPX}} \mathrm{ROH} + \mathrm{GSSG} + \mathrm{H_2O} \quad (1)$$

$$\mathrm{GSSG} + \mathrm{NADPH} + \mathrm{H^+} \xrightarrow{\mathrm{GSH\,reductase}} 2\mathrm{GSH} + \mathrm{NADP^+} \quad (2)$$

at 37 °C and pH 7.0, varying one substrate concentration while another is fixed. All kinetic experiments were performed at 37 °C in 700 µl of the reaction solution containing 0.5–3.0 mM GSH, 0.2–2.0 mM ROOH, 50 mM PBS (pH 7.0), 1 mM EDTA, 0.25 mM NAPDH, 1 U of GSH reductase and 4 µM 6-TeCD. Background absorption of the noncatalytic reaction was run without mimic and is subtracted. Kinetic data are analyzed by double-reciprocal plotting.

Results

MD simulations and docking study

To understand the interaction between β-CD and H_2O_2, *t*-BuOOH and CuOOH, the complexes of β-CD with the three ROOH are generated by the Insight II/Affinity module, and the binding 3D conformation of the three complexes are described in Fig. 1. From Fig. 1A, we can see that the H_2O_2 are loosely in the cavity of β-CD. From Fig. 1B, we can see that hydroxyl of *t*-BuOOH is located near the narrow side of β-CD, and a portion of one methyl of *t*-BuOOH is outside of β-CD cavity. In Fig. 1C, the CuOOH are full buried in the β-CD cavity. Compared complex of CuOOH-β-CD with other two complexes, we can see that the size and shape of β-CD cavity are matching for that of the CuOOH.

Binding ability is identified by the total interaction energy between β-CD and each ROOH. The total interaction energy, including van-der-Waals and electrostatic energies were shown in Table 1. From Table 1, we can see that the CuOOH-β-CD complex has favorable total interaction energy of –28.07 kcal mol^{-1}, the van-der-Waals and electrostatic energies are –24.92 and –3.15 kcal mol^{-1}, respectively. And the *t*-BuOOH-β-CD complex has worse

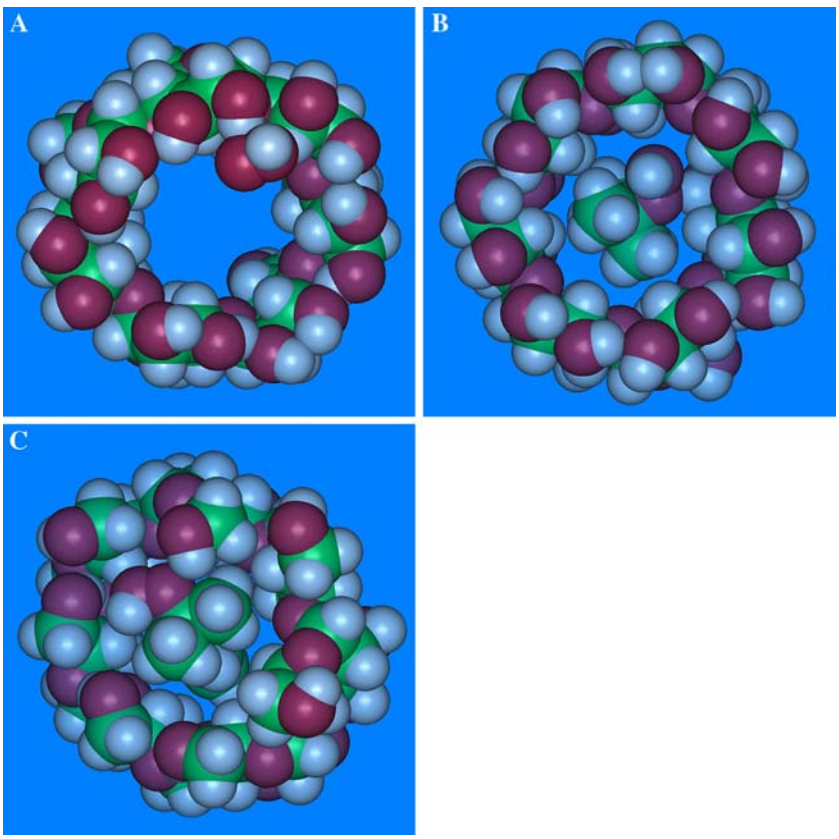


Fig. 1 Most stable conformation computed for complexes in a sphere of TIP3P water molecules with radius 20 Å, at pH 7.0 and 310 K. All views are from the narrow (primary hydroxyl) rim. (**A**) Complex of β-CD and H_2O_2, (**B**) Complex of β-CD and *t*-BuOOH, (**C**) Complex of β-CD and CuOOH

Table 1 The total energy (E_{total}), van-der-Waals energy (E_{vdw}) and electrostatic energy (E_{ele}) between ROOH (H_2O_2, *t*-BuOOH, CuOOH) and *β*-CD

ROOH	E_{vdw} (kcal mol^{-1})	E_{ele} (kcal mol^{-1})	E_{total} (kcal mol^{-1})
H_2O_2	–2.24	–2.83	–5.07
t-BuOOH	–12.68	–3.71	–16.39
CuOOH	–24.92	–3.15	–28.07

total interaction energy of –16.39 kcal mol^{-1}, the van-der-Waals and electrostatic energies of –3.71 and –12.68 kcal mol^{-1}, than that of the CuOOH-*β*-CD complex. The H_2O_2-*β*-CD complex has the worst total interaction energy of –5.07 kcal mol^{-1}, the van-der-Waals and electrostatic energies of –2.24 and –2.83 kcal mol^{-1}, respectively among in the three complexes. These results suggest that the abilities of *β*-CD to bind ROOH are as following order: CuOOH > *t*-BuOOH > H_2O_2.

Kinetics of 6-TeCD

Double-reciprocal plots (Figs. 2, 3) of initial rate versus substrate concentration at all the individual concentration revealed a family of parallel lines, which is characterized of a ping-pong mechanism [22] same as natural GPX [21]. The relevant steady-state equation (Eq 3) for the peroxidase reaction is:

$$\nu_0/[E]_0 = \frac{k_{max}[GSH] \times [ROOH]}{(K_{ROOH}[GSH] + K_{GSH}[ROOH] + [GSH] \times [ROOH])} \quad (3)$$

Where v_0 is the initial reaction rate, $[E]_0$ is the initial enzyme mimic concentration, k_{max} is a pseudo-first-order rate constant and K_{ROOH} and K_{GSH} are the Michaelis-Menten constants (K_m) for the ROOH and GSH, respectively. The kinetic parameters for the enzymatic reactions between GSH and the ROOH substrates H_2O_2, *t*-BuOOH, and CuOOH are shown in Table 2. The K_{ROOH} vary in the order of $K_{CuOOH} < K_{t\text{-}BuOOH} < K_{H2O2}$ for the enzymatic reactions. The second-order rate constant values of the reaction of 6-TeCD with ROOH ($k = k_{max}/K_{ROOH}$) vary in the order of k (CuOOH) > k (*t*-BuOOH) > k (H_2O_2). These results show that 6-TeCD has different substrate binding affinity and substrate specificity for ROOH, and the preferred substrate is CuOOH too.

Discussion

The complexes of ROOH with *β*-CD were investigated with molecular docking and MD simulations. The results of molecular docking indicate that CuOOH fits well to the size and shape of the cavity of *β*-CD and the results are

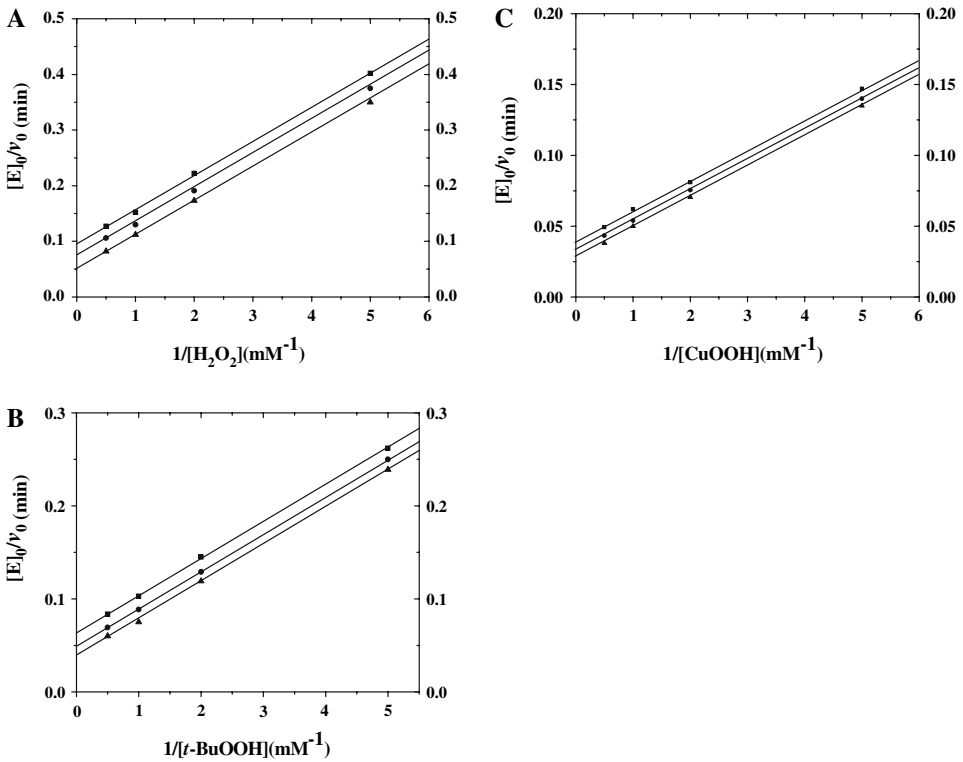


Fig. 2 Double-reciprocal plots of the initial velocity vs the concentration of the substrates. $[E]_0/v_0$ (min) vs 1/[ROOH] (mM^{-1}) for 4 μM 6-TeCD in 50 mM PBS, pH 7.0 and 37 °C, at [GSH] 0.5 (■), 1(●) and 3 mM (▲): (**A**) H_2O_2, (**B**) *t*-BuOOH, and (**C**) CuOOH

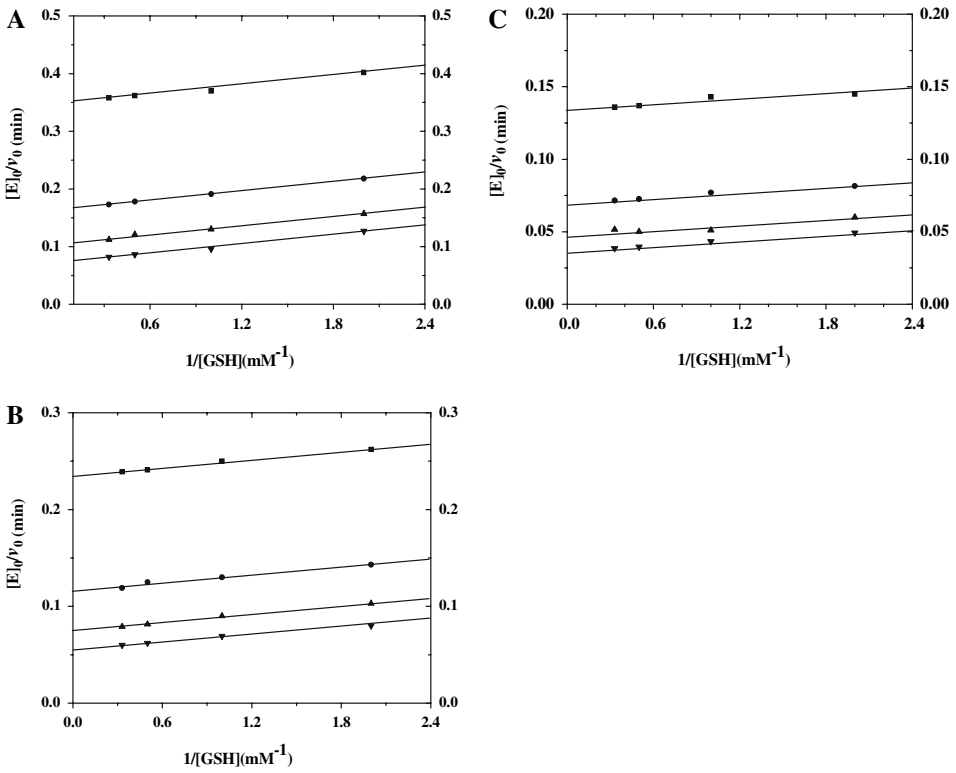


Fig. 3 Double-reciprocal plots of the initial velocity vs the concentration of the substrates. $[E]_0/v_0$ (min) vs 1/[GSH] (mM^{-1}) for 4 μM 6-TeCD in 50 mM PBS, pH 7.0 and 37 °C, at [ROOH] 0.2 (■), 0.5 (●), 1 (▲) and 2 mM (▼): (**A**) H_2O_2, (**B**) *t*-BuOOH, and (**C**) CuOOH

Table 2 Kinetic parameters of the 6-TeCD[a,b]

ROOH	$k_{max}(min^{-1})$	$K_{ROOH}(\mu M)$	$k_{max}/K_{ROOH}(M^{-1}\ min^{-1})$	$K_{GSH}(mM)$	$k_{max}/K_{GSH}(M^{-1}\ min^{-1})$
H_2O_2	23.7 ± 1.1	637 ± 23	$(3.70 \pm 0.26) \times 10^4$	1.45 ± 0.12	$(1.63 \pm 0.06) \times 10^4$
t-BuOOH	28.6 ± 0.4	413 ± 19	$(6.90 \pm 0.13) \times 10^4$	1.13 ± 0.07	$(2.53 \pm 0.14) \times 10^4$
CuOOH	37.6 ± 0.7	226 ± 11	$(1.67 \pm 0.34) \times 10^5$	0.81 ± 0.08	$(4.65 \pm 0.09) \times 10^4$

[a] Reactions were carried out in 50 mM PBS, pH 7.0, at 37 °C

[b] The data in the table were obtained from the plots in Fig. 2 and Fig. 3 and presented as means ± SD

well consistent with results of our latest work [13]. And the results of MD simulations also show that the favorable total interaction energy of CuOOH-*β*-CD is higher than that of *t*-BuOOH-*β*-CD and H_2O_2-*β*-CD. Consequently, in our miniature enzyme model, cavity of *β*-CD as a binding site, that provided maximum hydrophobic interaction with a substrate to form complexes, fits the aryl group of the bound substrate and the CuOOH could take advantage of the binding site of 6-TeCD well.

A kinetic parameter comparison (Table 2) was obtained from kinetic analyses of 6-TeCD using a variety of structurally distinct ROOH, such as H_2O_2, *t*-BuOOH, and CuOOH. Because the K_m is giving us information about the substrate binding power of the enzyme, a high K_m indicates a low affinity, and vice versa. Therefore the CuOOH is the most preferred substrate. The results of experiments are well consistent with results of MD simulations. The steady-state kinetics together with different K_{ROOH} values and the variety of k_{max}/K_{ROOH} values suggest that 6-TeCD has substrate specificity for ROOH and catalytically different efficiency for ROOH, and the preferred substrate is CuOOH. All the above results demonstrate that there are two key factors to effect the specificity of 6-TeCD to ROOH. One is whether size and shape of ROOH match with that of hydrophobic cavity of *β*-CD. Another one is that there is favorable interaction energy between hydrophobic cavity of *β*-CD and ROOH, or not.

Conclusion

In conclusion, we demonstrated that the specificities of 6-TeCD to different ROOH could be evaluated using both MD simulations and molecular docking because their results are well consistent with the experimental data on assay of the kinetics of 6-TeCD. Therefore, MD

simulations and molecular docking could be applied to evaluation substrate specificity of small-molecule enzyme mimics, and may be helpful for further experimental investigations.

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References

1. Flohé, L.: The glutathione peroxidase reaction: Molecular basis of the antioxidant function of selenium in mammals. Curr. Top. Cell. Regul. **27**, 473–478 (1985)
2. Tappel, A.L.: Selenium-glutathione peroxidase: Properties and synthesis. Curr. Top. Cell. Regul. **24**, 87–97 (1984)
3. Reich, H.J., Jasperse, C.P.: Organoselenium chemistry. Redox chemistry of selenocysteine model systems. J. Am. Chem. Soc. **109**, 5549–5553 (1987)
4. Ganther, H.E., Kraus, R.J.: Oxidation states of glutathione peroxidase. Methods Enzymol. **107**, 593–602 (1984)
5. Mugesh, G., Singh, B.H.: Synthetic organoselenium compounds as antioxidants: glutathione peroxidase activity. Chem. Soc. Rev. **29**, 347–357(2000)
6. Nogueira, C.W., Zeni, G., Rocha, J.B.: Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem. Rev. **104**, 6255–6285 (2004)
7. Mugesh, G., du Mont, W.W.: Structure-activity correlation between natural glutathione peroxidase (GPx) and mimics: a biomimetic concept for the design and synthesis of more efficient GPx mimics. Chemistry **7**, 1365–1370 (2001)
8. Mugesh, G., du Mont, W.W., Sies, H.: The Chemistry of Biologically Important Synthetic Organoselenium Compounds. Chem. Rev. **101**, 2125–2180 (2001)
9. Rekharsky, M.V., Inoue, Y.: Complexation thermodynamics of cyclodextrins. Chem. Rev. **98**, 1875–1917 (1998)
10. Szejtli, J.: Introduction and general overview of cyclodextrin chemistry. Chem. Rev. **98**, 1743–1753 (1998)
11. Breslow, R., Dong, S.D.: Biomimetic reactions catalyzed by cyclodextrins and their derivatives. Chem. Rev. **98**, 1997–2011 (1998)
12. Murakami, Y., Kikuchi, Ji.I., Hisaeda, Y., Hayashida, O.: Artificial enzymes. Chem. Rev. **96**, 721–758 (1996)
13. Dong, Z., Liu, J., Mao, S., Huang, X., Luo, G., Shen, J.: A glutathione peroxidase mimic 6,6-ditellurobis (6-Deoxy-β-cyclodextrin) with high substrate specificity. J. Inclu. Phenom. Macrocyc. Chem. **56**, 179–182 (2006)
14. Engman, L., Stern, D., Cotgreave, I.A., Andersson, C.M.: Thiol peroxidase activity of diaryl ditellurides as determined by a proton NMR method. J. Am. Chem. Soc. **114**, 9737–9743 (1992)
15. Dong, Z., Liu, J., Mao, S., Huang, X., Yang, B., Ren, X., Luo, G., Shen, J.: Aryl thiol substrate 3-carboxy-4-nitrobenzenethiol strongly stimulating thiol peroxidase activity of glutathione peroxidase mimic 2,2'-ditellurobis(2-Deoxy-β-cyclodextrin). J. Am. Chem. Soc. **126**, 16395–16404 (2004)
16. InsightII, Version 98.0, Accelrys Inc. San Diego, USA, (1998)
17. Betzel, C., Saenger, C., Hingerty, B.E., Brown, G.M.: Topography of cyclodextrin inclusion complexes, part 20. Circular and flip-flop hydrogen bonding in .beta.-cyclodextrin undecahydrate: a neutron diffraction study. J. Am. Chem. Soc. **106**, 7545–7557 (1984)
18. Jorgensen, W.L., Chandrasekhar, J., Madura, J.D., Impey, R.W., Klein, M.L.: Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. **79**, 926–935 (1983)
19. Discover3 User Guide, Accelrys Inc., San Diego, (1999)
20. Affinity User Guide, Accelrys Inc., San Diego, USA (1999)
21. Flohé, L., Loschen, G., Günzler, W.A., Eichele, E.: Glutathione peroxidase, V. The kinetic mechanism. Hoppe Seylers Z. Physiol. Chem. **353**, 987–999 (1972)
22. Bell, I.M., Hilvert', D.: Peroxide dependence of the semisynthetic enzyme selenosubtilisin. Biochemistry **32**, 13969–13973 (1993)