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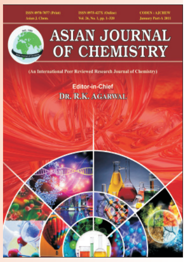

Investigation of Solvent-Dependent Catalytic Behaviour of Hydrophobic Guest Artificial Glutathione Peroxidase

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To reveal the relation between the catalytic rate of artificial glutathione peroxidase (GPx) and the property of solvent used in the determination of catalytic behaviour of glutathione peroxidase (ADA-Te-ADA) was investigated. Ethanol, DMSO, DMF and CH_3CN were selected as the co-solvent in the determination of catalytic rates. It was proved that ADA-Te-ADA exhibited the typical solvent-dependent catalytic behaviour. Moreover, compared with other co-solvents, the higher catalytic rate was observed when polar protic solvent (ethanol) was used. It suggested that the polar protic solvent was the appropriate co-solvent for the catalytic activity assay of hydrophobic artificial glutathione peroxidase. The strong polarity of polar aprotic solvent played an important role in the enhancement of glutathione peroxidase catalytic activity. This study explains the understanding of the catalytic behavior of hydrophobic guest artificial glutathione peroxidase when co-solvent was used.

Keywords: Artificial enzymes, Biomimetics, Enzyme activity, Glutathione peroxidase, Catalytic behaviour.

INTRODUCTION

Glutathione peroxidase (GPx, Ec.1.11.1.9) is an important selenium-containing enzyme among the antioxidative enzyme system. Glutathione peroxidase functions to protect various living organism from aerobic oxidative stresses by catalyzing the reduction of reactive oxygen species (ROS) using glutathione (GSH) as reducing substrate[1]. Usually, overproduced reactive oxygen species lead to many human oxidative stress-related diseases[2,3]. As the member of antioxidative defense system, GPx checks the overproduced ROS. Owing to its biologically crucial role, some artificial GPxs have been designed based on macromolecular scaffolds[4,5].

Among artificial GPxs with antioxidative catalytic ability constructed previously, artificial GPxs based on small molecules scaffolds have attracted more attentions[6-8]. The accurately catalytic elements of GPx with designable structure can be anchored to small molecules artificial GPx[7]. Recently, using the small molecules artificial GPx as building block, self-assembled supramolecular artificial GPxs are prepared[9]. Generally, the determination of the catalytic activity of small molecules artificial GPx and the construction of the supramolecular self-assembled artificial GPx are achieved in solvent mixture. However, up to now, the investigation of relation between the catalytic rate of artificial GPx and the property of solvent mixture is less reported, which has largely limited the further development of novel supramolecular self-assembled artificial GPx. Therefore, the elucidation of relation between the catalytic rate of artificial GPx and the property of solvent mixture is still a significant goal.

Therefore, to meet such significant challenge, a hydrophobic guest artificial GPx (ADA-Te-ADA) was employed and the catalytic behaviour of it was investigated. This method highlights the further development of novel supramolecular self-assembled artificial GPx using hydrophobic GPx as building block.

EXPERIMENTAL

Cumene hydroperoxide (CUOOH), NaH_2PO_4, Na_2HPO_4, methanol were purchased from J&K scientific Ltd and were used without further purification. 3-Carboxyl-4-nitrobenzenethiol (TNB) was synthesized from 5,5'-dithiobis(2-nitrobenzoic acid) as described previously[10]. ADA-Te-ADA was used as endowed from liu's group. The structure of ADA-Te-ADA was determined by Bruker 300 MHz spectrometer using a TMS proton signal as the internal standard (^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.09 (t, 2 H, $-COOCH_2$), 2.66 (t, 2 H, $-TeCH_2$), 2.07 (m, 2 H, $-CH_2-$), 2.01 (s, 3 H, adamantane), 1.88 (s, 6 H, adamantane),1.71 (s, 6 H, adamantane)). UV-visible spectra were obtained using a pgeneral T6 UV-visible spectrophotometer. The buffer pH values were determined with a METTLER TOLEDO 320 pH meter.

Determination of GPx activity in solvent mixture of PBS and ethanol: The catalytic activity was assayed according to a modified method reported by Wu and Hilvert[11]. The typical assay process was shown as follows: The reaction was carried out at 25 °C in a 1 mL quartz cuvette, 700 µL solvent mixture of PBS and ethanol and 100 µL of the catalyst (ADA-Te-ADA) (0.025 mM) were added and then 100 µL of the 3-carboxyl-4-nitro-benzenethiol solution(1 mM) was added. The mixture in the quartz cuvette was pre-incubated at the 25 °C for 3 min. Finally, the reaction was initiated by the addition of 100 mL of cumene hydroperoxide (2 mM) and the absorption decrease of 3-carboxyl-4-nitro-benzenethiol at 410 nm ($\varepsilon_{410} = 13600$ $M^{-1}cm^{-1}$. pH = 7) was monitored using a Pgeneral T6 UV-visible spectrophotometer. Appropriate control of the non-enzymatic reaction was performed and was subtracted from the catalyzed reaction. Each initial rate was measured at least 5 times and calculated from the first 5-10 % of the reaction.

Determination of GPx activity in solvent mixture of PBS and other co-solvent: The catalytic activity was assayed according to the process mentioned above except ethanol was replaced by other co-solvent.

Determination of the GPx catalytic rate influenced by ethanol: Typically, the volume ratios of PBS:ethanol used in the determination of the GPx catalytic rate were shown as follows: 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9.

Determination of the GPx catalytic rate influenced by other co-solvent: The catalytic activity was assayed according to the process mentioned above except ethanol was replaced by other co-solvent.

RESULTS AND DISCUSSION

Determination of GPx catalytic activity of ADA-Te-ADA: Herein, as shown in Fig.1, ADA-Te-ADA was selected as the typical hydrophobic artificial GPx. The catalytic behaviour of it was investigated. The structure of ADA-Te-ADA was illustrated in Fig.1. It was clearly that several hydrophobic groups presented in ADA-Te-ADA, such as adamantane, $-TeCH_2-$, $-CH_2-$ and so on. The solubility of ADA-Te-ADA in water was poor. Therefore, the catalytic property of ADA-Te-ADA was investigated using ethanol, DMSO, DMF, CH_3CN, as co-solvent, respectively. Typically, the catalytic activity of ADA-Te-ADA for the reduction of cumene hydroperoxide by 3-carboxyl-4-nitro-benzenethiol was evaluated according to the modified method reported by Wu and Hilvert[11] using 3-carboxyl-4-nitro-benzenethiol as a glutathione (GSH) alternative (Fig. 1). The absorption decrease of 3-carboxyl-4-nitro-benzenethiol at 410 nm ($\varepsilon_{410} = 13600\ M^{-1}\ cm^{-1}$, pH = 7) was monitored. Typically, the curves of the absorption decrease *vs.* time in the catalytic reduction using ethanol as co-solvent were depicted in Fig. 2. Compared with curve a of the absorption decrease without catalyst, the traditional small molecule artificial GPx PhSeSePh slightly enhanced the absorption decrease (curve c, $\upsilon_0 = 0.019\ \mu M\ min^{-1}$). However, when ADA-Te-ADA was used under the same condition, a remarkable rate enhancement (curve c, $\upsilon_0 = 4.16\ \mu M\ min^{-1}$) was observed. This observation proved that ADA-Te-ADA exhibited more excellent catalytic ability than traditional PhSeSePh. Additionally, the highest catalytic rates were observed when different co-solvents were used, which were given in Table-1.

Determination of GPx catalytic rate influenced by co-solvent: Herein, the solvent mixture consisted of PBS and co-solvent was employed as assay solution to determine the GPx catalytic rate. The ratio of PBS to co-solvent was fixed to 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9, respectively. Typically, GPx catalytic rate influenced by increasing added ethanol was investigated. The plotting of the catalytic reaction rate against the volume ratio of PBS to co-solvent was given in Fig. 3. Fig. 3a showed that the catalytic reaction rate of ADA-Te-ADA increased to some extent with ethanol increasing added. The highest value (4.16 µM min^{-1}) was obtained when the volume ratio was 6:4. However, the catalytic reaction rate largely decreased when the volume ratio increased further. Additionally, the similarly catalytic behaviours were also observed when DMSO, DMF and CH_3CN were used as co-solvent based on Fig. 3 b, c, d.

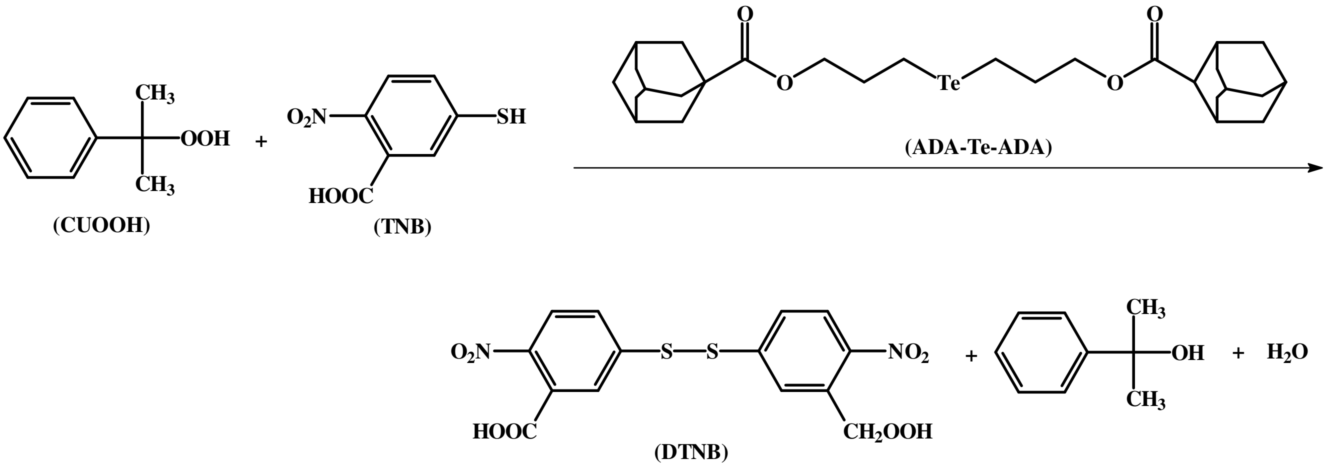

Fig. 1. Determination of GPx catalytic rates of ADA-Te-ADA for the reduction of cumene hydroperoxide (CUOOH) using 3-carboxyl-4-nitro-benzenethiol (TNB) as substrates

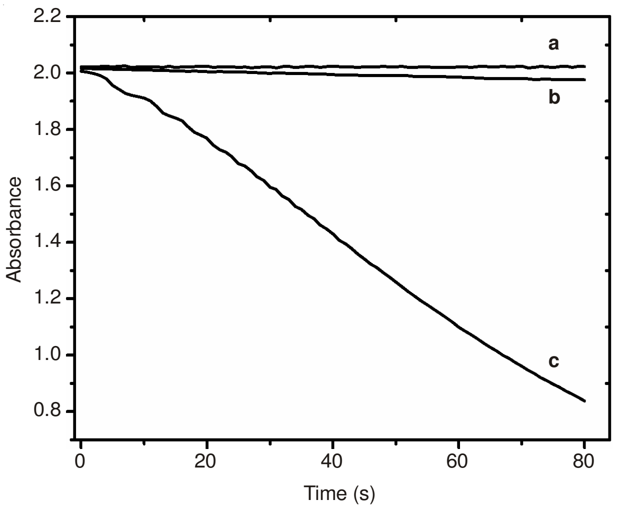

Fig. 2. Plots of absorbance vs. time in the catalytic reduction of cumene hydroperoxide (2 mM) by 3-carboxyl-4-nitro-benzenethiol (1 mM) at pH 7 and 25 °C using ethanol as co-solvent (PBS: ethanol, v:v, 6:4) (a) No catalyst, (b) 0.025 mM PhSeSePh, (c) ADA-Te-ADA (0.025 mM)

TABLE-1
INITIAL RATES (υ_0) AND ACTIVITIES FOR THE REDUCTION OF CUMENE HYDROPEROXIDE (2 mM) BY 3-CARBOXYL-4-NITRO-BENZENETHIOL (1 mM) IN THE PRESENCE OF THE ADA-Te-ADA (0.025 mM) AT pH 7 AND 25 °C

Co-solvent	PBS: co-solvent (v:v)	υ_0 (mM min^{-1})[a]
Ethanol	6:4	4.16±0.21
DMSO	7:3	3.77±0.26
DMF	7:3	3.27±0.14
CH_3CN	6:4	3.02±0.17

[a] Initial rate of reaction was corrected for the spontaneous oxidation. And the concentration of catalyst is 0.025 mM and assuming one molecule catalytic center (tellurium moiety) as one active site of enzyme

As ADA-Te-ADA consisted of several hydrophobic groups, we speculated that the interesting phenomena of catalytic rate increasing to some extent with the volume ratio going up was driving from the change of solubility of ADA-Te-ADA in solvent mixture. So the better solubility of ADA-Te-ADA was favorable for the homogeneous phase system consisted ADA-Te-ADA and substrates. And the highest value was exhibited when the appropriate solubility of ADA-Te-ADA and substrates was endowed. Furthermore, the possible reason for the decreased catalytic reaction rate might endow from the hydrophobic driving force. It is noted from previous report that the hydrophobic driving force might result in the conformation change of hydrophobic dendrimer-based artificial GPx[8]. Such conformation change could alter the substrate selectivity of artificial GPx. Similarly, ADA-Te-ADA and substrate cumene hydroperoxide were hydrophobic molecules. So PBS was poor solvent for the hydrophobic artificial enzyme ADA-Te-ADA and the hydrophobic substrate cumene hydroperoxide. Therefore, the solvent mixture with more PBS might drive ADA-Te-ADA and cumene hydroperoxide matched closely in the catalytic reaction. When more co-solvent was added and PBS was less, the match of ADA-Te-ADA and cumene hydroperoxide could not be achieved appropriately as the hydrophobic driving force from PBS was poor although the ADA-Te-ADA dissolved well. So it is concluded that only

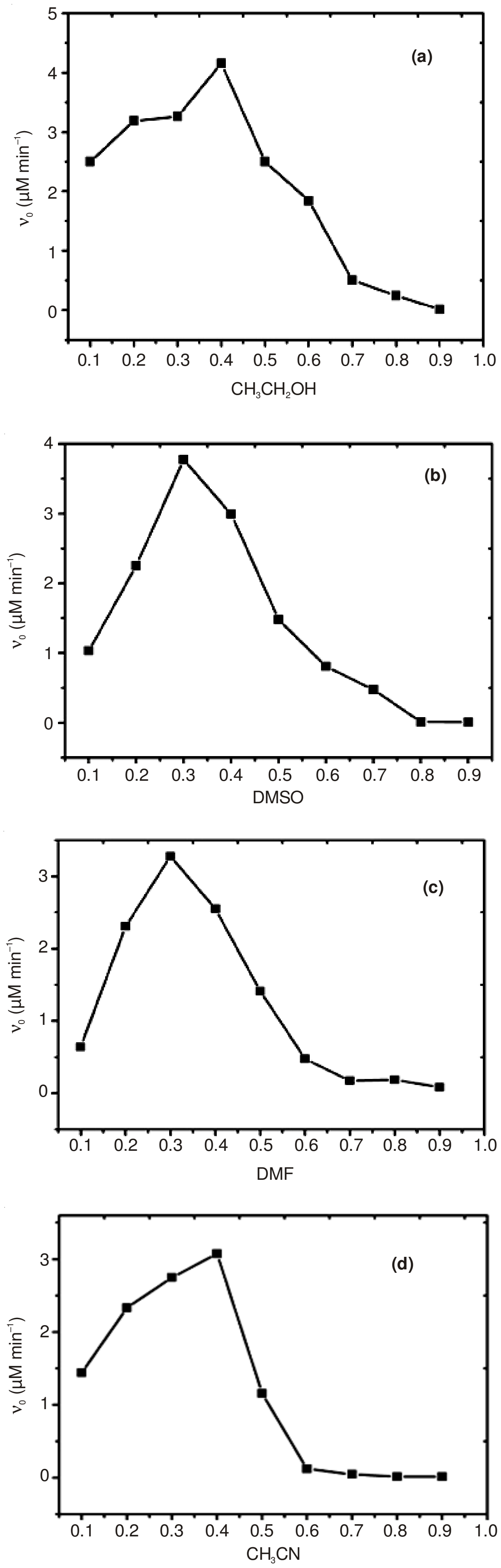

Fig. 3. Plots of catalytic reaction rates υ_0 against the volume ratios of co-solvent: (a) ethanol; (b) DMSO; (c) DMF; (d) CH_3CN

co-solvent added with appropriated ratio was favourable for the enhancement of GPx catalytic ability.

Discussion of the solvent-dependent catalytic behaviour of ADA-Te-ADA: Moreover to illustrate and compare the highest initial rates (υ_0) obtained when various co-solvent with appropriate ratio was used (Fig. 4). It was shown that the sequence of the highest initial rates obtained using co-solvents was like this: A(ethanol) > B(DMSO) > C(DMF) > D(CH_3CN). It is known that among the four co-solvents, ethanol was polar protic solvent. DMSO, DMF and CH_3CN were polar aprotic solvent. Combining the sequence of the highest initial rates and the properties of four co-solvents, it is concluded that the ethanol was the most suitable co-solvent for the enhancement of catalytic activity as the polar protic solvent. Among the three polar aprotic solvents, the polarity sequence was as follows: DMSO > DMF > CH_3CN. As shown in Fig. 4, the sequence of the highest initial rates of the three polar aprotic solvents was in accordance with polarity sequence. It is suggested that the strong polarity of polar aprotic solvent was favorable for the enhancement of GPx catalytic activity. Therefore, we can draw a conclusion that polar protic solvent is the suitable co-solvent for the enhancement of catalytic activity. And the strong polarity of polar aprotic solvent plays an important role in the enhancement of GPx catalytic activity. This conclusion might function as the basement for the understanding of the catalytic behaviour of hydrophobic guest artificial GPx.

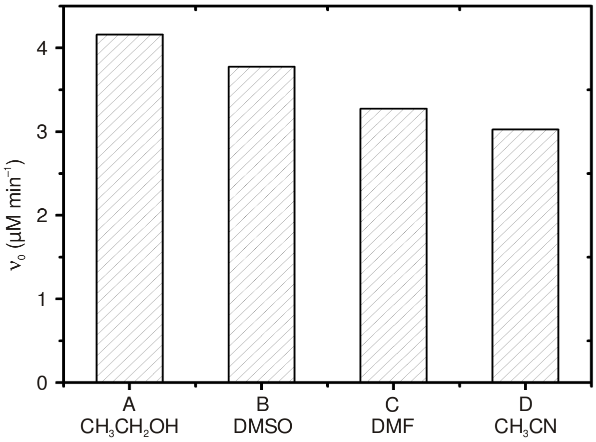

Fig. 4. The highest initial rates (υ_0) obtained using various co-solvent; (a) ethanol; (b) DMSO; (c) DMF; (d) CH_3CN

Conclusion

Herein, a hydrophobic guest molecule, ADA-Te-ADA, was selected as the hydrophobic artificial GPx. And the relation between the catalytic rate of it and the property of solvent was investigated. It was proved that ADA-Te-ADA exhibited the typical solvent-dependent catalytic behaviour when different volume ratios of co-solvents were respectively added. Moreover, the higher catalytic rate was observed when polar protic solvent (ethanol) was used compared with other co-solvents, which suggested that polar protic solvent was the appropriate co-solvent for the assay of hydrophobic artificial GPx. And the strong polarity of polar aprotic solvent plays an important role in the enhancement of GPx catalytic activity.

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