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Investigation of Solvent-Dependent Catalytic Behaviour of Hydrophobic Guest Artificial Glutathione Peroxidase Using Cumene Hydroperoxide and 4-Nitrobenzenethiol as Substrates

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The catalytic behaviour of a hydrophobic guest glutathione peroxidase (ADA-Te-ADA) was detailed investigated using cumene hydroperoxide and 4-nitrobenzenethiol as substrates. The relation between the catalytic rate of artificial glutathione peroxidase and the property of solvent used in the determination of catalytic rate was revealed. Herein, C_2H_5OH, DMSO, DMF and CH_3CN were selected as the co-solvents in the determination of catalytic rates. The typical solvent-dependent catalytic behaviour of ADA-Te-ADA was exhibited. Especially, the higher catalytic rate was observed when polar protic solvent (ethanol) was used compared with other co-solvents. It suggested that polar protic solvent was the appropriate co-solvent for the assay of catalytic activity of hydrophobic artificial glutathione peroxidase. Additionally, the strong polarity of polar aprotic solvent plays an important role in the enhancement of glutathione peroxidase catalytic activity. This study suited well for the understanding of the catalytic behaviour of hydrophobic guest artificial glutathione peroxidase

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

INTRODUCTION

Among the antioxidative enzyme system, glutathione peroxidase (GPx, Ec.1.11.1.9) is an important selenium-containing enzyme. Glutathione peroxidase functions to protect various living organism from aerobic oxidative stresses by catalyzing the reduction of reactive oxygen species using glutathione (GSH) as reducing substrate[1]. Commonly, over produced reactive oxygen species (ROS) lead to many human oxidative stress-related diseases[2,3]. Glutathione peroxidase can clear the overproduced reactive oxygen species as one member of the family of the antioxidative enzymes. Owing to its biologically crucial role, some artificial glutathione peroxidases have been designed based on macromolecular scaffolds in our group[4,5].

During the past decades, various artificial glutathione peroxidases with antioxidative catalytic ability were constructed. Especially, artificial glutathione peroxidases based on small molecules scaffolds have attracted more attentions[6-8]. The accurately catalytic elements of glutathione peroxidase with designable structure can be anchored to small molecule artificial glutathione peroxidases[7]. Recently, self-assembled supramolecular artificial glutathione peroxidases are prepared using the small molecule artificial glutathione peroxidases as building blocks[9]. Generally, the construction of the supramolecular self-assembled artificial glutathione peroxidase is achieved in solvent mixture. Determination of the catalytic activity of small molecule artificial glutathione peroxidases is also carried out in solvent mixture. However, up to now, the investigation of relation between the catalytic rate of artificial glutathione peroxidase and the property of solvent mixture is few reported, which has largely limited the further development of novel supramolecular self-assembled artificial glutathione peroxidase. Therefore, the elucidation of relation between the catalytic rate of artificial glutathione peroxidase and the property of solvent mixture is still a significant goal.

Therefore, to meet such significant challenge, a hydrophobic guest artificial glutathione peroxidase (ADA-Te-ADA) was employed. The catalytic behaviour of ADA-Te-ADA was investigated using cumene hydroperoxide and 4-nitrobenzenethiol as substrates. These substrates have been proved to be more excellent and appropriate substrates for the determination of catalytic activity of glutathione peroxidase[4,5,9]. This method highlights the further development of novel supramolecular self-assembled artificial glutathione peroxidase using hydrophobic glutathione peroxidase as building block.

EXPERIMENTAL

Cumene hydroperoxide (CUOOH), 4-nitrobenzenethiol (NBT), NaH_2PO_4, Na_2HPO_4, ethanol were purchased from

J&K Scientific Ltd. and were used without further purification. ADA-Te-ADA was synthesized according to the previous reported[10]. The structure of ADA-Te-ADA was determined as: ^{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 glutathione peroxidase activity in solvent mixture of PBS and co-solvents: The catalytic activity was assayed according to a modified method reported by Wu and Hilvert[11]. The typical assay process of glutathione peroxidase activity in solvent mixture of PBS and ethanol was shown as follows: The reaction was carried out at 25 °C in a 1 mL quartz cuvette, 700 μL solvent mixture of PBS, ethanol and 100 μL of the catalyst (ADA-Te-ADA) (0.025 mM) were added and then 100 μL of the 4-nitrobenzenethiol solution (1 mM) was added. The mixture in the quartz cuvette was pre-incubated at 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 4-nitrobenzenethiol at 410 nm (ε_{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. The glutathione peroxidase activities in solvent mixture of PBS and other co-solvents were assayed similarly except ethanol was replaced by other co-solvents.

Determination of the glutathione peroxidase catalytic rates influenced by co-solvents: The volume ratios of PBS: ethanol used in the determination of the glutathione peroxidase catalytic rate were considered as: 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8. The catalytic activities influenced by other co-solvents were assayed similarly except ethanol was replaced by other co-solvents.

RESULTS AND DISCUSSION

Determination of the glutathione peroxidase catalytic activity of ADA-Te-ADA: Herein, to reveal the relation between the catalytic rate of artificial glutathione peroxidase and the property of solvent mixture, ADA-Te-ADA was selected as the typical hydrophobic artificial glutathione peroxidase (Fig. 1). It was clear that several hydrophobic groups presented in ADA-Te-ADA, such as adamantane, -$TeCH_2$-, -CH_2- and so on. Therefore, the solubility of ADA-Te-ADA in water was poor. Thus, the catalytic property of ADA-Te-ADA was investigated using ethanol, DMSO, DMF, CH_3CN, as co-solvents, respectively. Typically, the catalytic activity of ADA-Te-ADA for the reduction of cumene hydroperoxide by 4-nitrobenzenethiol was evaluated according to the modified method reported by Wu and Hilvert[11] using 4-nitrobenzenethiol as a glutathione (GSH) alternative (Fig. 1). Compared with the traditional small molecule artificial glutathione peroxidase PhSeSePh (v_0 = 0.019 μM × min^{-1}), a remarkable rate enhancement was observed when ADA-Te-ADA was functioned as artificial glutathione peroxidase under the conditions of different solvent mixture (Table-1). 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.

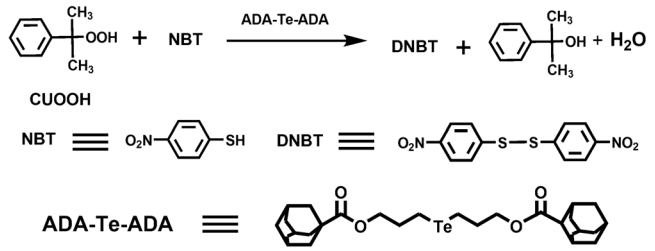

Fig. 1. Determination of glutathione peroxidase catalytic rates of ADA-Te-ADA for the reduction of CUOOH using NBT as substrate

TABLE-1
INITIAL RATES (v_0) AND ACTIVITIES FOR THE REDUCTION OF CUMENE HYDROPEROXIDE (2 mM) BY 4-NITRO-BENZENETHIOL (1 mM) IN THE PRESENCE OF ADA-Te-ADA (0.025 mM) AT pH 7 and 25 °C

Co-solvent	PBS:co-solvent (v:v)	v_0 (mM min^{-1})[a]
C_2H_5OH	6:4	4.21 ± 0.35
DMSO	6:4	2.71 ± 0.22
DMF	5:5	2.55 ± 0.13
CH_3CN	6:4	3.22 ± 0.28

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

Determination of the glutathione peroxidase catalytic rate influenced by co-solvent: Herein, the solvent mixture consisted of PBS and co-solvent was employed as assay solution to determine the glutathione peroxidase 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, respectively. Typically, glutathione peroxidase catalytic rate influenced by increasing added ethanol was investigated. By plotting the catalytic reaction rate against the volume ratio of PBS to co-solvent (Fig. 2). From Fig. 2a, we noted that the catalytic rate of ADA-Te-ADA increased to some extent with ethanol increasing added. And the highest value (4.21 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-solvents (Fig. 2b-d).

Considering that ADA-Te-ADA consisted of several hydrophobic groups, it is speculated that the interesting phenomena of catalytic rate increasing to some extent with the volume ratio going up was derived from the change of solubility of ADA-Te-ADA in solvent mixture. Therefore, 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 achieved. Furthermore, the possible reason for the decreased catalytic reaction rate might be endowed from the hydrophobic driving force. It was noted that the hydrophobic driving force might result in the conformation change of hydrophobic dendrimer-based artificial glutathione peroxidase[8]. Thus, the change of conformation could alter the substrate selectivity of artificial

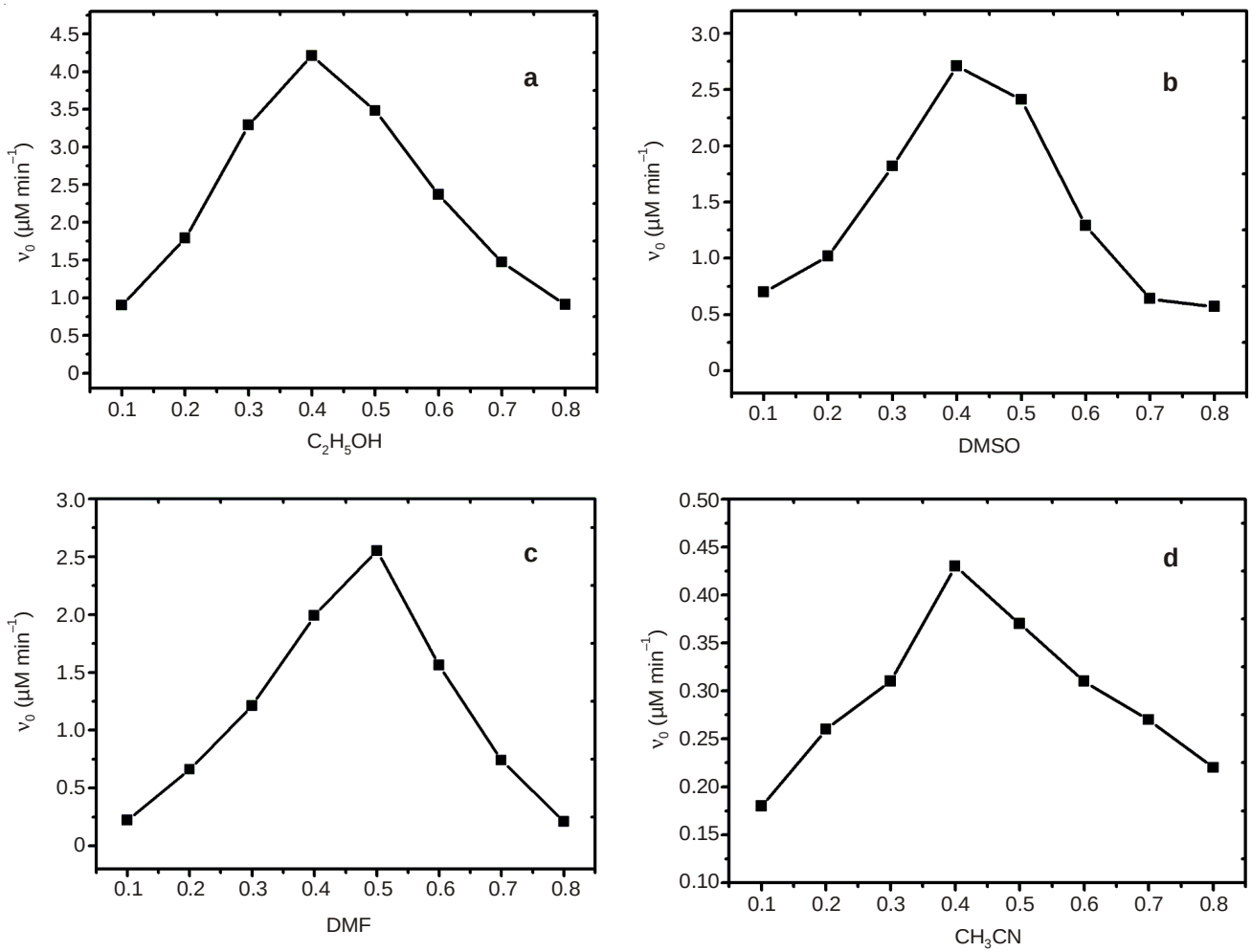

Fig. 2. Plots of catalytic rates v_0 against the volume ratios of co-solvent: a: C_2H_5OH; b: DMSO; c: DMF; d: CH_3CN

glutathione peroxidase. Similarly, ADA-Te-ADA and substrate cumene hydroperoxide were hydrophobic molecules. Considering that PBS was poor solvent for the hydrophobic molecules, the solvent mixture with more PBS might drive ADA-Te-ADA and cumene hydroperoxide matched closely in the catalytic reaction process. Therefore, the match of ADA-Te-ADA and cumene hydroperoxide could not be achieved appropriately when more co-solvent and less PBS were added. So we can conclude that only co-solvent added with appropriated ratio was favorable for the enhancement of glutathione peroxidase catalytic ability.

Solvent-dependent catalytic behaviour of ADA-Te-ADA: Fig. 3 illustrates and compare the highest initial rates. It was shown that the sequence of the highest initial rates obtained using different co-solvents was like this: A(C_2H_5OH) > B(DMSO) > C(DMF) > D(CH_3CN). As we known, among the four co-solvents, C_2H_5OH was polar protic solvent. DMSO, DMF and CH_3CN were polar aprotic solvent. Fig. 3 concluded that C_2H_5OH was the most suitable co-solvent for the enhancement of catalytic activity as the polar protic solvent. Additionally, among the three polar aprotic solvents, the polarity sequence was as follow: DMSO > DMF > CH_3CN. Obviously, the sequence of the highest initial rates related to the three polar aprotic solvents was in accordance with polarity sequence. It suggested that the strong polarity of polar aprotic solvent

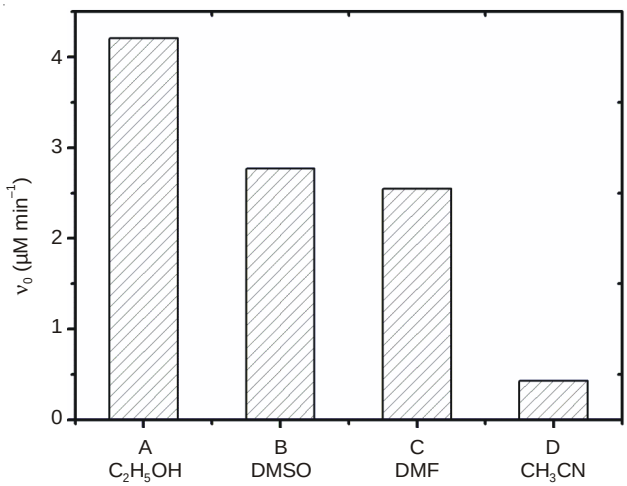

Fig. 3. Highest initial rates (v_0) obtained using different co-solvents. (A) C_2H_5OH; (B) DMSO; (C) DMF; (D) CH_3CN

was favorable for the enhancement of glutathione peroxidase catalytic activity. Therefore, we can draw a conclusion that polar protic solvent is the suitable co-solvent for the enhancement of catalytic activity. The strong polarity of polar aprotic solvent plays an important role in the enhancement of glutathione peroxidase catalytic activity. This conclusion might function as the basement for the understanding of the catalytic

behaviour of hydrophobic guest artificial glutathione peroxidase.

Conclusion

Herein, the relation between the catalytic rate of ADA-Te-ADA 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 glutathione peroxidase. And the strong polarity of polar aprotic solvent plays an important role in the enhancement of glutathione peroxidase catalytic activity.

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