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Investigation of Solvent-Dependent Catalytic Behaviour of Hydrophobic Guest Artificial Glutathione Peroxidase Using H_2O_2 and 3-Carboxyl-4-nitrobenzenethiol as Substrates

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The investigation of the catalytic behaviour of a hydrophobic guest artificial glutathione peroxidase (GPx) (**ADA-Te-OH**) was carried out employing H_2O_2 and 3-carboxyl-4-nitrobenzenethiol (TNB) as substrates. The relation between the catalytic rate of **ADA-Te-OH** and the property of solvent used in the determination of catalytic activity was revealed. Typically, the co-solvents including ethanol, DMSO, DMF and CH_3CN were employed in the determination of catalytic rates. It indicated that **ADA-Te-OH** exhibited the typical solvent-dependent catalytic behaviour. Especially, 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 embodies well understanding of the catalytic behaviour of hydrophobic guest artificial glutathione peroxidase.

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

INTRODUCTION

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

Among various artificial glutathione peroxidases with antioxidative catalytic ability constructed during the past decades, artificial glutathione peroxidases based on small molecules scaffolds have attracted more attentions. It is because that the accurately catalytic elements of glutathione peroxidase with designable structure can be anchored to small molecule artificial glutathione peroxidases[6-8]. Recently, employing the small molecule artificial glutathione peroxidases as building blocks, self-assembled supramolecular artificial glutathione peroxidases are constructed[9]. Generally, both the construction of the supramolecular self-assembled artificial glutathione peroxidase and the determination of the catalytic activity of small molecule artificial glutathione peroxidases are carried out in solvent mixture. However, up to now, the investigation of relation between the property of solvent mixture and the catalytic rate of artificial glutathione peroxidase was less 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 challenge.

Therefore, to achieve such a significant goal, a hydrophobic guest artificial glutathione peroxidase (**ADA-Te-OH**) was employed. And the catalytic behaviour of **ADA-Te-OH** was detailed investigated using H_2O_2 and 3-carboxyl-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]. Herein, this method highlights the further development of novel supramolecular self-assembled artificial glutathione peroxidase using hydrophobic glutathione peroxidase as building block.

EXPERIMENTAL

Hydrogen peroxide, 3-carboxyl-4-nitrobenzenethiol (TNB), NaH_2PO_4, Na_2HPO_4, ethanol were purchased from J&K Scientific

Ltd. and were used without further purification. **ADA-Te-OH** was synthesized according to the previous reported[10]. The structure of **ADA-Te-OH** was determined as this. (1H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.09 (t, 2 H), 3.71 (t, 2 H), 2.72 (t, 2 H), 2.66 (t, 2 H), 2.07 (m, 2 H), 2.00 (s, 3 H), 1.88 (s, 6 H), 1.71 (s, 6 H)). UV-vis spectra were obtained using a Shimadzu 2600 UV-visible-NIR 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 Hilvert and Wu[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 and ethanol and 100 μL of the catalyst (**ADA-Te-OH**)(0.025 mM) were added and then 100 μL of the 3-carboxyl-4-nitrobenzenethiol 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 H_2O_2 (2 mM) and the absorption decrease of 3-carboxyl-4-nitrobenzenethiol at 410 nm (ε_{410} = 13600 M^{-1} cm^{-1}. pH = 7.0) was monitored by a Shimadzu 2600 UV-visible-NIR 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 glutathione peroxidase catalytic rates influenced by co-solvents: Typically, the volume ratios of PBS:ethanol used in the determination of the glutathione peroxidase catalytic rate were shown as follows: 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 glutathione peroxidase catalytic activity of ADA-Te-OH: Herein, **ADA-Te-OH** was selected as the typical hydrophobic artificial glutathione peroxidase to reveal the relation between the catalytic rate of artificial glutathione peroxidase and the property of solvent mixture. Fig. 1 showed that the several hydrophobic groups presented in **ADA-Te-OH**, such as adamantane, -$TeCH_2$-, -CH_2-, *etc*. Therefore, the solubility of **ADA-Te-OH** in water was poor. So the catalytic property of **ADA-Te-OH** was investigated using ethanol, DMSO, DMF, CH_3CN, as co-solvents, respectively. Typically, the catalytic activity of **ADA-Te-OH** for the reduction of H_2O_2 by 3-carboxyl-4-nitro-benzenethiol was evaluated according to the modified method reported by Hilvert and Wu[11] using 3-carboxyl-4-nitrobenzenethiol as an alternative of glutathione (Fig. 1). Compared with the catalytic rate of the traditional small molecule artificial glutathione peroxidase PhSeSePh (v_0 = 0.019 μM min^{-1}), a remarkable rate enhancement was observed under the conditions of different solvent mixture when **ADA-Te-OH** was functioned as artificial glutathione peroxidase (Table-1). This observation suggested that **ADA-Te-OH** exhibited more excellent catalytic ability than traditional PhSeSePh. Additionally, the highest catalytic rates were observed when different co-solvents were used and they were given in Table-1.

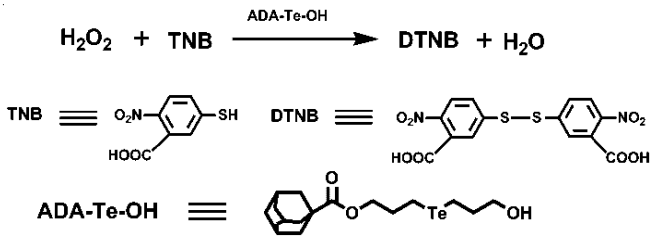

Fig. 1. Determination of glutathione peroxidase catalytic rates of **ADA-Te-OH** for the reduction of H_2O_2 using 3-carboxyl-4-nitrobenzenethiol as substrate

TABLE-1
INITIAL RATES (v_0) AND ACTIVITIES FOR THE REDUCTION OF H_2O_2 (2 mM) BY 3-CARBOXYL-4-NITROBENZENETHIOL (1 mM) IN THE PRESENCE OF THE **ADA-Te-OH** (0.025 mM) AT pH 7.0 AND 25 °C

Co-solvent	PBS:Co-solvent (v:v)	v_0 (mM min^{-1})[a]
Ethanol	6:4	3.68 ± 0.22
DMSO	6:4	2.59 ± 0.18
DMF	5:5	1.72 ± 0.13
CH_3CN	6:4	0.95 ± 0.08

[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

Determination of glutathione peroxidase catalytic rate influenced by co-solvent: Herein, the solvent mixture consisted of PBS and co-solvent was used 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. Vividly, by plotting the catalytic reaction rate against the volume ratio of PBS to co-solvent, Fig. 2 was given. Typically, glutathione peroxidase catalytic rate influenced by increasing added ethanol was investigated and shown in Fig. 2a. It is noted that the catalytic rate of **ADA-Te-OH** increased to some extent with ethanol increasing added. And the highest value (3.68 μM×min^{-1}) was obtained when the volume ratio was 6:4. However, the catalytic reaction rate largely went down when the volume ratio increased further. Additionally, the similar catalytic behaviours were also observed when DMSO, DMF and CH_3CN were used as co-solvents based on Fig. 2b-d.

Considering that **ADA-Te-OH** 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 derived from the change of solubility of **ADA-Te-OH** in solvent mixture. Therefore, the better solubility of **ADA-Te-OH** was favourable for the homogeneous phase system consisted **ADA-Te-OH** and substrates. And the highest value was exhibited when the appropriate solubility of **ADA-Te-OH** and substrates was achieved. Furthermore, the possible reason for the decreased catalytic reaction rate might be endowed from the decreasing of PBS. It was noted that the polar environment endowed from PBS played an important role in maintaining the high catalytic rate and were

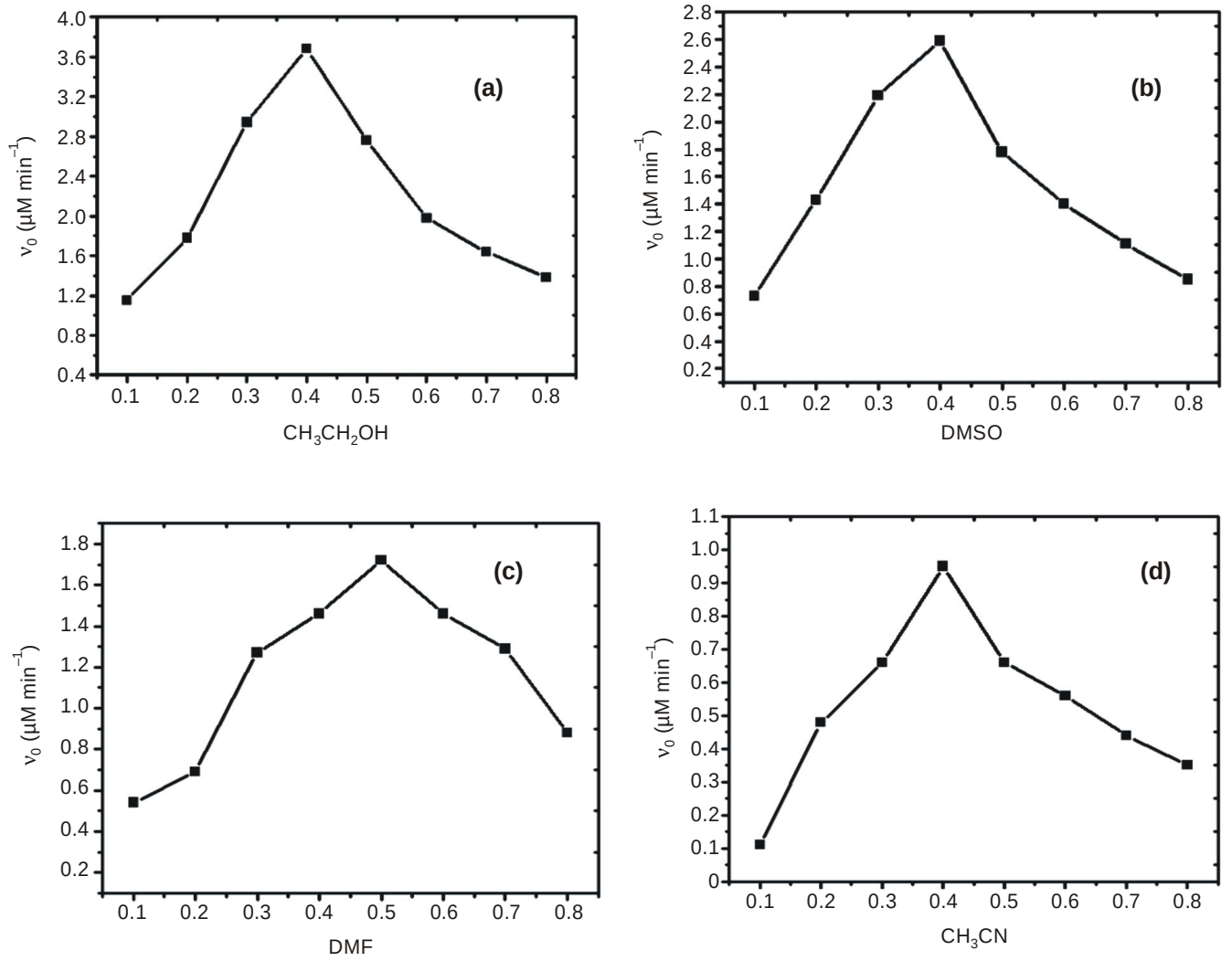

Fig. 2. Plots of catalytic rates v_0 against the volume ratios of co-solvent: (a) ethanol; (b) DMSO; (c) DMF; (d) CH_3CN

favourable for the accomplishment of catalytic process[8]. Thus, it is concluded that the decreased catalytic reaction rate might be endowed from the decreasing of PBS. So we can draw a conclusion that only co-solvent added with appropriated ratio was favourable for the enhancement of glutathione peroxidase catalytic ability.

Solvent-dependent catalytic behaviour of ADA-Te-OH: Moreover, it was shown in Fig. 3 that the sequence of the highest initial rates obtained using different co-solvents was like this: A(ethanol) > B(DMSO) > C(DMF) > D(CH_3CN). As it is known that among the four co-solvents, ethanol was polar protic solvent. DMSO, DMF and CH_3CN were polar aprotic solvent. Fig. 3 concluded that ethanol 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 was favourable for the enhancement of glutathione peroxidase catalytic activity. Therefore, a conclusion has been drawn 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 glutathione peroxidase catalytic activity. This conclusion might function as the basement for the understanding of the catalytic behaviour of hydrophobic guest artificial glutathione peroxidase.

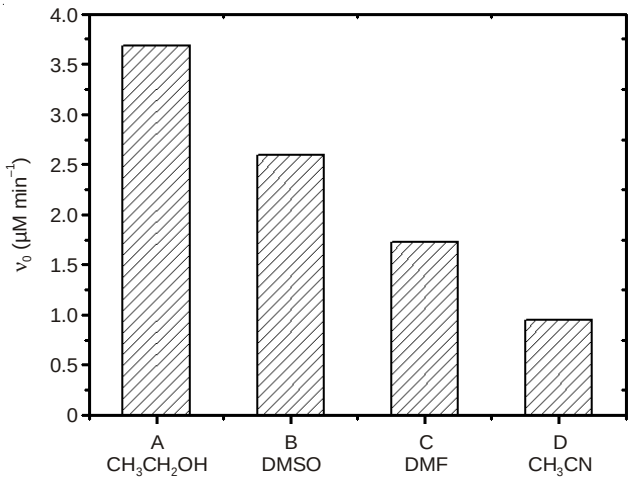

Fig. 3. Highest initial rates (v_0) obtained using different co-solvents (A) ethanol; (B) DMSO; (C) DMF; (D) CH_3CN

Conclusion

Herein, the investigation of the relation between the catalytic rate of **ADA-Te-OH** and the property of solvent was carried out. It was proved that **ADA-Te-OH** exhibited the typical solvent-dependent catalytic behaviour when different volume ratios of co-solvents were respectively added. Moreover, 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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