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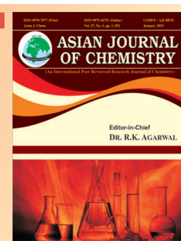

Solvent-Dependent Catalytic Behavior of Telluride-Containing Guest Artificial Glutathione Peroxidase Using Cumene Hydroperoxide and 3-Carboxyl-4-nitrobenzenethiol as Substrates

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To reveal the solvent-dependent catalytic behaviour of a hydrophobic telluride-containing guest artificial glutathione peroxidase (ADA-Te-OH), the catalytic rates were investigated using cumene hydroperoxide and 4-nitrobenzenethiol as substrates. Herein, ethanol, DMSO, DMF and CH_3CN were selected as the co-solvents in the determination of catalytic rates. Significantly, the typical solvent-dependent catalytic behaviour of ADA-Te-OH was observed. 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. This study 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

As an important selenium-containing enzyme of the family of the antioxidative enzyme system, glutathione peroxidase (GPx, Ec.1.11.1.9) functions to protect various living organism from aerobic oxidative stresses by catalyzing the reduction of ROS using glutathione (GSH) as reducing substrate[1]. Commonly, glutathione peroxidase can over produced reactive oxygen species 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[4,5].

Recently, 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 can be anchored to small molecule artificial glutathione peroxidases[7]. Thus, 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 peroxidases is achieved in solvent mixture. And the determination of the catalytic activity of them is also carried out in solvent mixture. However, up to now, the investigation on relation between the catalytic rate of artificial glutathione peroxidase and the property of solvent mixture is less reported. 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 (3,3'-telluro-*bis*(propane-3,1-diyl) adamantane carboxylate, ADA-Te-OH) was employed. Considering that hydroperoxide (CUOOH) and 3-carboxyl-4-nitrobenzenethiol (TNB) have been proved to be more excellent and appropriate substrates for the determination of catalytic activity of glutathione peroxidase[4,5,9]. The catalytic behaviour of ADA-Te-OH was investigated using hydroperoxide and 3-carboxyl-4-nitrobenzenethiol as substrates. This method highlights the further development of novel supramolecular self-assembled artificial glutathione peroxidase using hydrophobic glutathione peroxidase as building block.

EXPERIMENTAL

Hydroperoxide, 4-nitrobenzenethiol, 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 (^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-visible 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 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 hydroperoxide (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 using 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 the 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; 1:9. 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-OH: Herein, to reveal the relation between the catalytic rate of artificial glutathione peroxidase and the property of solvent mixture, ADA-Te-OH was selected as the typical hydrophobic artificial glutathione peroxidase (shown in Fig. 1). Typically, the structure of ADA-Te-OH was illustrated in Fig. 1. It was clearly shown that several hydrophobic groups are presented in ADA-Te-OH, such as adamantane, -$TeCH_2$-, -CH_2-, *etc*. Therefore, the solubility of ADA-Te-OH in water was poor. Thus, 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 hydroperoxide by 3-carboxyl-4-nitrobenzenethiol was evaluated according to the modified method reported by Hilvert and Wu[11] using 3-carboxyl-4-nitrobenzenethiol as a glutathione (GSH) alternative (Fig. 1). Compared with the traditional small molecule artificial glutathione peroxidase PhSeSePh (ν_0 = 0.019 µM min^{-1}), a remarkable rate enhancement was observed when ADA-Te-OH was used as artificial glutathione peroxidase under the conditions of different solvent mixture (Table-1). This observation proved 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 (Table-1).

TABLE-1
INITIAL RATES (ν_0) AND ACTIVITIES FOR THE REDUCTION OF CUOOH (2 mM) BY 3-CARBOXYL-4-NITROBENZENETHIOL (1 mM) IN THE PRESENCE OF THE ADA-Te-OH (0.025 mM) AT pH 7 AND 25 °C

Co-solvent	PBS:co-solvent (v:v)	ν_0(mM min^{-1})[a]
Ethanol	6:4	2.23 ± 0.12
DMSO	7:3	2.05 ± 0.08
DMF	7:3	1.67 ± 0.15
CH_3CN	6:4	1.57 ± 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 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; 2:8,1:9, respectively. Typically, glutathione peroxidase catalytic rate influenced by increasing added ethanol was investigated (Fig. 2 a). From Fig. 2 a, we noted that the catalytic rate of ADA-Te-OH increased to some extent with ethanol increasing added. And the highest value (2.23 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 similarly catalytic behaviours were also observed when DMSO, DMF and CH_3CN were used as co-solvents based on Fig. 2 b, c, d.

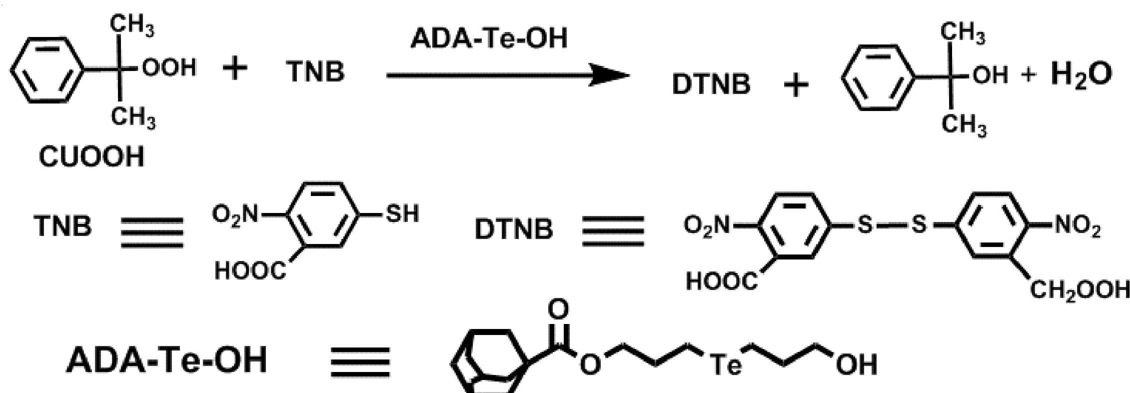

Fig. 1. Determination of glutathione peroxidase catalytic rates of ADA-Te-OH for the reduction of hydroperoxide using 3-carboxyl-4-nitrobenzenethiol as substrate

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

Considering that ADA-Te-OH 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 resulted from the change of solubility of ADA-Te-OH in solvent mixture. Therefore, the better solubility of ADA-Te-OH was favorable for the homogeneous catalytic process. 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 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]. Therefore, the change of conformation could alter the substrate selectivity of artificial glutathione peroxidase. Similarly, ADA-Te-OH and substrate hydroperoxide were hydrophobic molecules. And the match of ADA-Te-OH and hydroperoxide could not be achieved appropriately when more co-solvent and less PBS were added. Thus, it is concluded 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-OH: Moreover, Fig. 3 was given to vividly illustrate and compare the highest catalytic rates. It was shown that the sequence of the highest initial rates obtained using different co-solvents

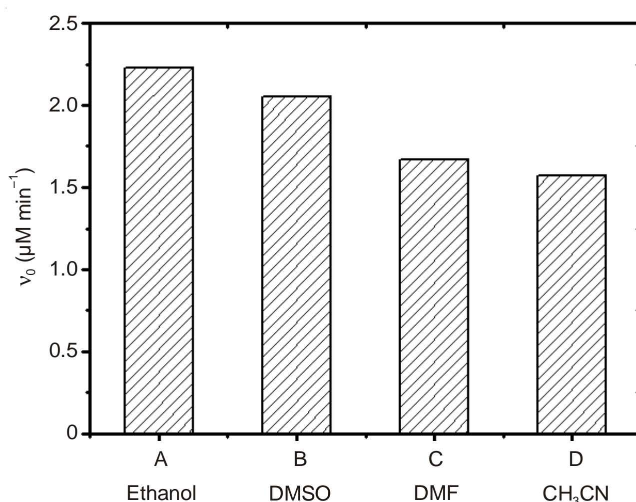

Fig. 3. Highest initial rates (v_0) obtained using different co-solvents. (A) Ethanol; (B) DMSO; (C) DMF; (D) CH_3CN

was like this: A(ethanol) > B(DMSO) > C(DMF) > D(CH_3CN). 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. It was noticeable that the sequence of the highest initial rates related to the three polar aprotic solvents was in accordance with polarity sequence, which suggested that the strong polarity of polar aprotic solvent was favorable for the enhancement of glutathione peroxidase catalytic activity. Therefore, a conclusion drawn 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 under-standing of the catalytic behaviour of hydrophobic guest artificial glutathione peroxidase.

Conclusion

Herein, the relation between the catalytic rate of ADA-Te-OH and the property of solvent was investigated. It was suggested that ADA-Te-OH 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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