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^{1}H NMR Study on the Inclusion Complex of Glutathione with a Glutathione Peroxidase Mimic, 2,2′-ditelluro-bridged β-cyclodextrins

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Abstract

The complex formation of 2, 2′-ditelluro-bis(β-cyclodextrin) (2-TeCD) with glutathione (GSH) was investigated in D_2O at room temperature by ^{1}H nuclear magnetic resonance (^{1}H NMR) technique. The association constant and stoichiometry between GSH and 2-TeCD was determined from the chemical shifts dependence of the H5 proton in GSH on the concentration of 2-TeCD. The stoichiometry of the inclusion complex was determined by the molar method to be of 2:1 host-to-guest. 2-TeCD showed higher affinity toward GSH than β-cyclodextrin (β-CD). This may be attributed the reason that 2-TeCD which possesses dual hydrophobic cavities in a close vicinity enhances GSH binding ability through the cooperative binding of two cavities. The formation of the $(2\text{-TeCD})_2$/GSH complex was one of the reason that 2-TeCD showed higher glutathione peroxidase (GPX) activity than Ebselen.

Introduction

Glutathione peroxidase (GPX) is a selenoenzyme composed of four identical subunits of 21,000 Da which catalyzes the reduction of H_2O_2 and other hydroperoxides by glutathione (GSH) (Figure 1a) [1, 2]. In many tissues, such as the lens of the eye, glutathione peroxidase is the major defense against hydroperoxide [3]. However, GPX has some shortcomings, such as instability, poor availability and high molecular weight, which have limited its therapeutic use [4]. In recent years, there were increasing interests in mimicking the functions of this important antioxidant enzyme (GPX) not only for elucidating catalytic mechanism but also for potential pharmaceutical application, and several efforts had been made to produce synthetic selenium/tellurium compounds which can mimic the properties of glutathione peroxidase [5–7]. Cyclodextrins (CDs) can act as artificial enzymes [8–11]. CDs, like enzymes, possess specific catalytic properties and form 'enzyme-substrate' complexes in which the hydrophobic moiety of the substrate, in particular any aromatic group, is enclosed in the CD cavity [12, 13]. CDs and their tellurium analogues had all been demonstrated to catalyze the reduction of hydroperoxides (ROOH) in the presence of thiols [7, 14]. Possessing dual hydrophobic cavities in a close vicinity, bridged cyclodextrin dimers had been demonstrated to greatly enhance the original molecular binding ability of the parent cyclodextrin through the cooperative binding of one guest molecule in the closely located two cyclodextrin cavities [15–18], which provided an excellent model system mimicking the substrate-specific interaction of enzymes [19, 20]. The generation of specific binding ability for thiol substrate and correct incorporation of the functional selenium/tellurium groups should be critical approaches for the construction of an effective GPX model [21]. By cooperating of both recognition by cyclodextrin and catalysis by ditelluro moiety, cyclodextrin-based enzyme model 2, 2′-ditelluro-bis(β-cyclodextrin) (2-TeCD) (Figure 1c) had been reported to act as a GPX mimic [14, 22].

^{1}H NMR spectroscopy was one of the most useful techniques for investigating the stability and stoichiometry of complexes, particularly in the solution. NMR spectra obtained from most CD complexes represent concentration-weighted averages since exchange between the free and complexed guest molecule was usually fast in the NMR time scale. Therefore, to determine association constants with CDs, nonlinear fitting of chemical shift changes as a function of concentration (the so-called NMR titration) was usually performed [23, 24]. In the present study, the stoichiometry and

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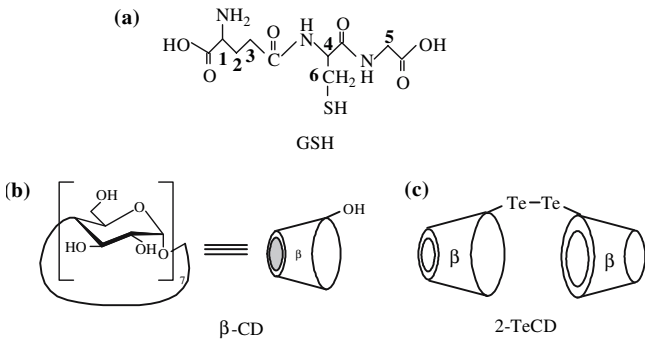


Figure 1. Schematic chemical structures of (a) GSH, (b) β-CD and (c) 2-TeCD.

stability constant of 2-TeCD with GSH were investigated in D_2O at room temperature by using concentration dependent ^1H NMR. In order to comparison, β-cyclodextrin (β-CD) (Figure 1b) was also studied by ^1H NMR method.

Experimental section

Chemicals

β-CD and GSH were purchased from Sigma-Aldrich Chemical Co. Both chemicals were used without any additional purification. 2-TeCD was synthesized and purified according to the literature [22]. The deuterium content of D_2O was 99.5%.

NMR spectra

D_2O was used as a solvent for all NMR measurements. All NMR experiments were performed on a Bruker AM-500 spectrometer (500 MHz) at ambient temperature using residual HOD as an internal standard (4.700 ppm).

Complexation studies

GSH was dissolved in D_2O (concentration of 10 mM was used). GSH solution was separated into two portions. A portion was used as a guest NMR sample, and the remainder was used to dissolve and to dilute the sample of the host, so that the concentration of GSH remained constant throughout the titration. Successive aliquots of the host solution were added to GSH sample, and ^1H NMR spectra were recorded after each addition. Samples for ^1H NMR measurements were prepared directly in an NMR tube by adding appropriate amounts of the host and a guest with a microsyringe.

Results and discussion

Figure 2 presented ^1H NMR spectra of (a) GSH, (b) β-CD and (c) the mixture of GSH and β-CD at the room temperature. The signals of the β-CD recorded in the

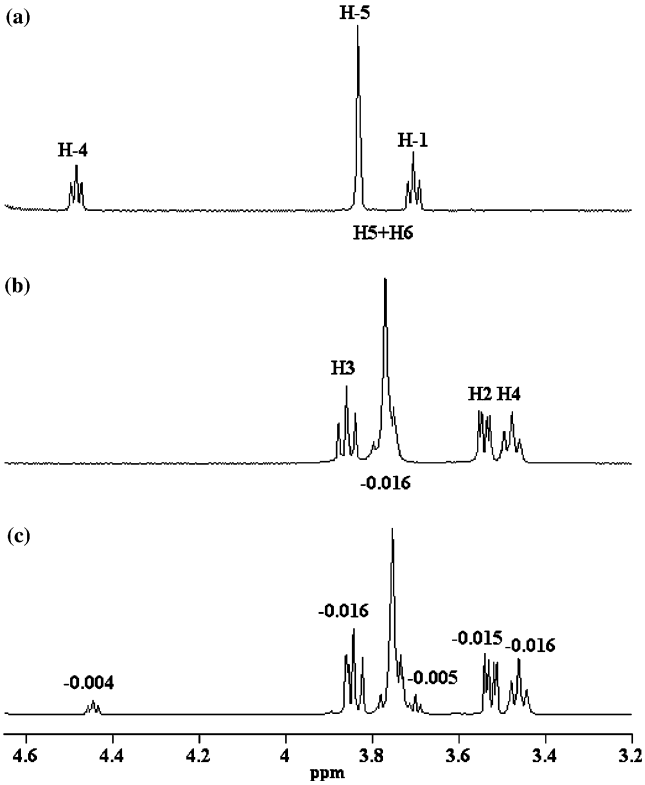


Figure 2. ^1H NMR spectra at room temperature in D_2O of (a) 10 mM GSH, (b) 10 mM β-CD and (c) the mixture of 10 mM β-CD and 10 mM GSH, respectively.

presence of GSH showed some differences with respect to those of β-CD alone. In particular, both the internal H3 and H5 protons of β-CD underwent upfield shifts of 0.016 ppm in the presence of equimolar amounts of GSH. It can be taken as an indication of the occurrence of a 'host-guest' interaction [23]. Outer protons of H2 and H4 also underwent upfield shifts, this may be attributed to interaction other than inclusion. Since GSH does not contain an aromatic side chain, the binding interaction of the (CD/GSH) complexes in solution should be dominated by hydrogen bonding, which is different from that of most complexes studied previously [25–27]. Comparison with alone GSH, the protons of GSH showed different degree upfield shifts after the addition of β-CD. The concretely chemical shifts of GSH protons were –0.005 (H1), –0.003 (H2 and H3), –0.004 (H6) and –0.005 (H6′) ppm, respectively. These slight while different degree signal shifts of GSH protons also confirmed the formation of the β-CD/GSH complex.

^1H NMR spectra of GSH in the presence of different amount of 2-TeCD were shown in Figure 3. With similar to the β-CD/GSH complex, the signals of GSH recorded in the presence of 2-TeCD showed some differences with respect to those of the free GSH, and the chemical shifts of GSH protons were enlarged with 2-TeCD increasing. When the molar of 2-TeCD was doubled to GSH, the chemical shifts of GSH protons were –0.008, 0.001, 0.182, –0.083, –0.008 ppm for H1, 2 or 3, 4, 5, 6′, respectively. Meanwhile, the peak of

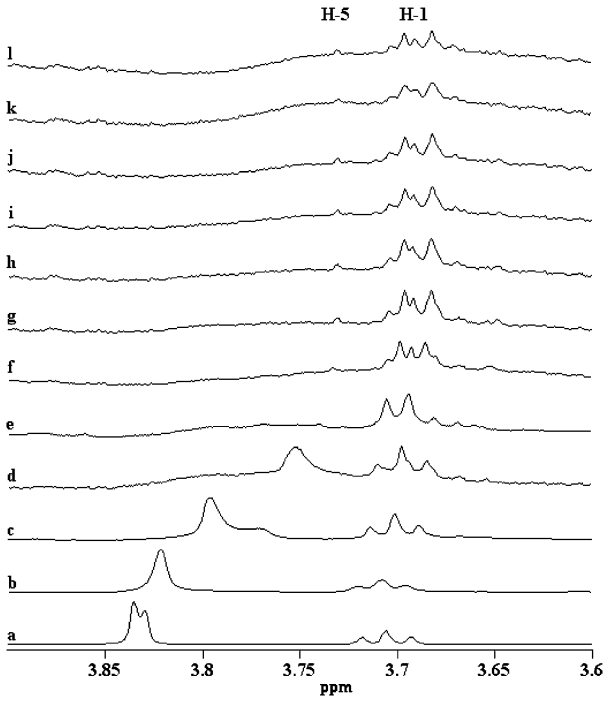


Figure 3. ^{1}H NMR spectra as a function of molar ratios of GSH/2-TeCD ranging from 1:0 (a) to 1:9 (l) at room temperature. The proton designations correspond to the structures shown in Figure 1.

proton 6 of GSH alone was one group in the region of 2.814–2.909 ppm. In the presence of 2-TeCD, this one group peak split into two groups in the region of 2.858–2.931 ppm and 3.188–3.256 ppm, respectively. The chemical shifts of GSH protons and significant split of H-6 of GSH sufficiently determined the appearance of a 'host-guest' interaction between 2-TeCD and GSH.

The experimental NMR spectra represented the concentration-weighted average of the spectra of GSH in water and that of it after included in the 2-TeCD cavities. Therefore, the association constant and stoichiometry could be calculated by the concentration dependence of the chemical shifts ($\Delta\delta$) values of the GSH. To extract such information, the differences of the resonance frequency of the H-5 proton were measured from the NMR spectra because the signal was well resolved and considerably intense. Figure 4 showed plots of the shift values of the H-5 signal of GSH *versus* the mole concentration ratio of 2-TeCD to GSH. The stoichiometry of the host to guest in the inclusion complex was determined to be 2:1 by the molar method.

To our knowledge, the study about the association constant of cyclodextrin with guest complex in 2:1 stoichiometry by NMR method was few. Therefore, based on the Ishizu study [28] which aimed at the solution structures of the 1:1 inclusion complexes of β-CD with (+)-catechin and (-)-Epicatechin by NMR method we deduced the formula that used to calculate the association constant of $(2\text{-TeCD})_2$/GSH complex with 2:1 stoichiometry. The detail of deduced process was introduced in following.

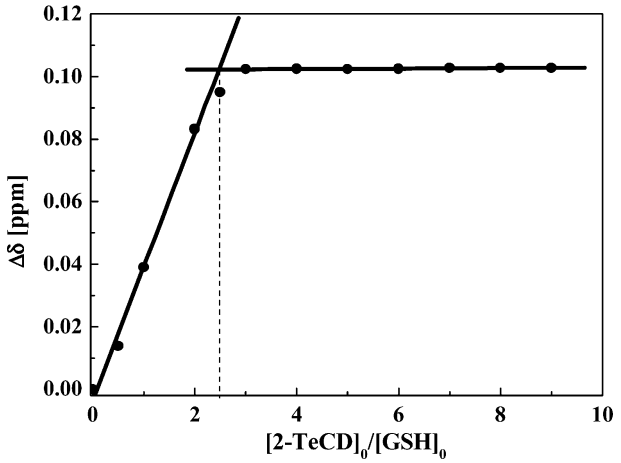


Figure 4. Plots of the shift values of the H5 signal of GSH by adding 2-TeCD *versus* the molar concentration ratio of 2-TeCD to GSH at room temperature.

The stability constant of the inclusion complex K_c (M^{-1}) was represented in equation (1), where [G], [H], and [H_2G] represented the mole concentration of GSH, 2-TeCD, and the $(2\text{-TeCD})_2$/GSH complex (mM), respectively, and $[G]_0$ and $[H]_0$ were respect to the initial concentration (mM) of guest and host. In equation (3), δ_{GSH}, δ_{H_2G} and δ_{obs} represented the chemical shift (ppm) of H-5 of GSH, the inclusion complex of $(2\text{-TeCD})_2$/GSH and the mixture of 2-TeCD with GSH in ^{1}H NMR spectra, respectively [29].

$$2H + G \xrightarrow{K_c} H_2G$$

$$K_c = \frac{[H_2G]}{[G][H]^2} = \frac{[H_2G]}{([G]_0 - [H_2G])([H]_0 - 2[H_2G])^2} \quad (1)$$

which on rearrangement transformed to

$$\frac{1}{K_c} = \left(\frac{[G]_0}{[H_2G]} - 1\right)([H]_0 - 2[H_2G])^2$$

$$\frac{1}{[G]_0^2 K_c} = \left(\frac{[H]_0}{[G]_0} - \frac{2[H_2G]}{[G]_0}\right)^2 \left(\frac{[G]_0}{[H_2G]} - 1\right) \quad (2)$$

θ was expressed as

$$\theta = \frac{[G]_0 - [G]}{[G]_0} = \frac{[H_2G]}{[G]_0}$$

The observed chemical shift (ppm) of H5 of GSH would be given by

$$\delta_{obs} = \delta_G(1 - \theta) + \delta_{H_2G}\theta$$

$$\theta = \frac{\delta_G - \delta_{obs}}{\delta_G - \delta_{H_2G}} = \frac{\Delta\delta_{obs}}{\Delta\delta_{H_2G}} \quad (3)$$

M was expressed as

$$M = \frac{[H]_0}{[G]_0} \quad (4)$$

Combining equations (2), (3), and (4) gave

$$\frac{1}{[G]_0^2 K_c} = \left(\frac{1}{\theta} - 1\right)(M - 2\theta)^2$$

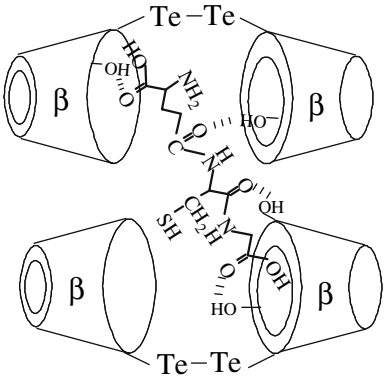
Which on rearrangement transformed to

$$M = 2\theta \pm \frac{1}{[G]_0}\sqrt{\frac{\theta}{(1-\theta)K_c}} \qquad (5)$$

Combining equations (5), (3), and (4) and transforming gave

$$[H]_0 = \frac{2\Delta\delta_{obs}}{\Delta\delta_{H_2G}}[G]_0 \pm \sqrt{\frac{\Delta\delta_{obs}}{(\Delta\delta_{H_2G} - \Delta\delta_{obs})K_c}} \qquad (6)$$

The K_c value at room temperature was estimated from the change in $\Delta\delta_{obs} = (\delta_G - \delta_{H_2G})$ *versus* increasing $[H]_0$ at constant $[G]_0$ using equation 6 (Figure 5). The K_c value for inclusion complex of $(2\text{-TeCD})_2$/GSH at room temperature was calculated to be $3.27 \times 10^4\ M^{-2}$ suggesting that 2-TeCD had a moderate ability to bind GSH. It had been demonstrated that strongly hydrophilic molecules were usually difficult, or only very weakly included into the hydrophobic cavity of cyclodextrin [30]. In some reported studies [25–27], it had been suggested that the interactions between CDs and small hydrophilic molecules in the aqueous solution were dominated by hydrogen bonding. Likewise, the binding interaction of $(2\text{-TeCD})_2$/GSH complex in solution may also dominated by hydrogen bonding, as illustrated in (Scheme 1). Such model can be confirmed by the chemical shift of GSH protons after binding with 2-TeCD. The carbonyl groups and mercapto group of GSH might form hydrogen bonds with the hydroxyl groups of 2-TeCD. The hydrogen bonding interaction between GSH and 2-TeCD induced large chemical shifts of neighbored protons, H4 ($\Delta\delta$ = 0.182 ppm) and H5 ($\Delta\delta$ = –0.083 ppm), which lead to the relative high association constant of $(2\text{-TeCD})_2$/GSH complex.



Scheme 1. Possible binding mode between 2-TeCD and GSH.

Although the current NMR data did not able to describe the detail conformation of the $(2\text{-TeCD})_2$/GSH complex, it could be sure that GSH could form complex with 2-TeCD. This was one of the crucial reasons that under the same conditions, the GPX activities of 2-TeCD for the reduction of H_2O_2 by GSH was at least 46 times more efficient than that of Ebselen [22, 31]. Being the chemical shifts of H5 proton of GSH fluctuated to the concentration of β-CD, the stoichiometry and stability constant of β-CD/GSH complex could not be obtained. This might be due to that the chemical shifts of H5 of GSH was simply averaged signals between free and complexed GSH. This indicated that the inclusion was too fast on the NMR time scale [32]. Differently viewed, this results illustrated that 2-TeCD which possesses dual hydrophobic cavities in a close vicinity enhanced GSH binding ability through the cooperative binding of two cavities.

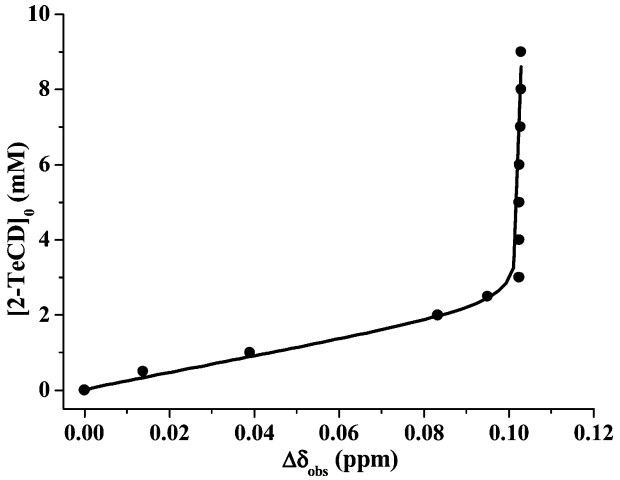


Figure 5. Curve-fitting analysis of the differential shift of H5 proton of GSH ($\Delta\delta_{obs}$) to calculate the complex stability constant (*Kc*) upon addition of 2-TeCD (0–9 mM) in D_2O at temperature.

Conclusions

We have carried out a simple but powerful ^1H NMR technique for characterizing the complex interaction between 2-TeCD and GSH. The association constant of $(2\text{-TeCD})_2$/GSH complex is calculated to be $3.27 \times 10^4\ M^{-2}$ with a stoichiometry of 2:1 host to guest based on NMR titration spectra, which shows that 2-TeCD has a moderate ability to bind GSH and the binding interaction was dominated by hydrogen bonding. It can be concluded that it is the complex formation between 2-TeCD and GSH that improved its GPX activity in catalyzing the reduction of hydrogen peroxide by GSH.

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