DPPH radical scavenging activity of selenocompounds: importance of selenium

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Abstract
To investigate the antioxidant property of several selenocompounds we synthesized, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined by ESR. In this study, the experimental conditions for ESR measurement were also examined. Phosphate-buffered saline (PBS) was a better solvent for DPPH than dimethylsulfoxide (DMSO) or ethanol to obtain the strong signal intensity and the high S/N ratio. Among absolute signal intensity, absolute signal area, relative signal intensity to MnO, and relative signal area to MnO, the most suitable parameter corresponding to the concentration of DPPH was absolute signal intensity, because the correlation coefficient between absolute signal intensity and DPPH concentration was the highest. In low-molecular-weight selenocompounds, diethyldithiocarbamate selenotrisulfide (DEDC-SeT) had almost the same degree of DPPH radical scavenging activity as reduced glutathione or dithiothreitol, which are reference antioxidant compounds. The activity of DEDC-SeT was higher than that of sodium diethyldithiocarbamate or its disulfide form. Four polysaccharide-selenocystamine (SeCyst) conjugates, heparin-cystamine (Hep-Cyst), pullulan-selenocystamine (Pul-SeCyst), laminarin-selenocystamine (Lam-SeCyst), fucoidan-selenocystamine (Fuc-SeCyst) had DPPH radical scavenging activity which was similar to that of ascorbic acid or reduced glutathione. The activity of Hep-Cyst was higher than that of heparin or heparin-cystamine conjugate which has sulfur instead of selenium in the molecule. These results suggest that selenium is involved in the antioxidant activity of DEDC-SeT and polysaccharide-SeCyst conjugates.

Keywords: selenium, selenocompound, DPPH, radical, antioxidant activity, ESR

Introduction
Antioxidant activity is one of the most important functions of selenium. The activity is considered to be dependent on the chemical forms of selenium. Glutathione peroxidase (GPx) has selenocysteine residues in its catalytic center where selenium is present as selenol. Though selenol in the low-molecular-weight compounds is unstable and readily oxidized to diselenide in the air as selenocysteine, it seems to be more stable in the high-molecular-weight compounds.

We have synthesized heparin-selenocystamine conjugate (Hep-SeCyst) which has selenol groups in the molecule and they were stable for months in the air. It was shown that they have antioxidant activity [1]. We have also synthesized the compounds with selenotrisulfide structure. The antioxidant activity of the selenotrisulfide compounds has not been investigated.

Ebselen is an antioxidant selenocompound which has GPx-like activity and reacts with peroxynitrite, a powerful oxidant [2]. However, it is shown by the spectrophotometric method that ebselen has little DPPH radical scavenging activity [3]. These results suggest the reactivity of a selenocompound with prooxidant radicals is different for each radical species.

To evaluate the antioxidant activity of several selenocompounds which include the compounds we synthesized and some other reference compounds,
DPPH radical scavenging activities were determined by ESR, which enables to measure the radical species directly.

Materials and Methods

1. Materials

DEDC-SeT was synthesized as described before [4]. Laminarin, fucoidan and pullulan were purchased from Nacalai Tesque Inc.. Molecular weight of these polysaccharides were 7000-8000, 113000-153000, and 50000-100000, respectively. The Hep-Cyst, Lam-SeCyst, and Fuc-SeCyst were prepared by the method described for Hep-SeCyst before [1]. Penicillamine selenotrisulfide (Pen-SeT) was prepared by the method of Nakagawa et al. [5]. These conjugates were stable for at least several months at 4°C. Ebselen was a gift from Daiichi Seiyaku Co., Ltd. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Nacalai Tesque, Inc. Other chemicals were reagent grade. Selenium and sulfur in the conjugates were determined by atomic absorption spectrometry and spectrophotometry, respectively.

2. Conditions for ESR measurement of DPPH

A JES-FR30 Free Radical Monitor (JEOL, Japan) with 100kHz modulation frequency and a microwave power of 4mW was used to record the ESR spectra. To compare the ESR signals of DPPH in different solutions, DPPH was dissolved in 10mM phosphate buffered saline, pH7.4 (PBS), DMSO, or ethanol at a final concentration of 100μM. In the preparation of PBS solution, one volume of 1mM DMSO solution of DPPH was diluted with 9 volumes of PBS. The DPPH signal intensity was not affected by DMSO concentration in the sample within 20%. Sample solution was packed in a glass capillary and it was put in the quartz glass cell. ESR signal intensity of DPPH and S/N ratio were observed for these solutions.

To select the most suitable practical ESR parameter to represent the concentration of DPPH, the correlation between the parameters, which are absolute signal intensity, absolute signal area, relative signal intensity to MnO, and relative signal area to MnO, and DPPH concentrations were analyzed.

3. DPPH radical scavenging activity of selenocompounds

After DPPH was incubated with low-molecular-weight selenocompounds or polysaccharide-SeCyst conjugates in PBS for 2 hours at 23°C, ESR signal intensity of DPPH was measured. The selenocompounds or reference compounds were dissolved in PBS containing 10%DMSO at a final concentration of 10 or 100μM.

DPPH radical scavenging activity of selenocompounds was represented as the reduced percentage of signal intensity in the presence of selenocompounds compared with the signal intensity observed in the absence of those compounds.

Results and Discussion

The highest value of ESR signal intensity (199.4) and the highest S/N ratio (90.6) were obtained when DPPH was dissolved in PBS (Fig.1, Table 1), and DPPH was dissolved in PBS in this study. The signal intensity of DPPH was comparatively stable for 2 hours at 23°C (room temperature), and 93% of the initial value was maintained after 2 hours (data not shown).

The correlation coefficients of ESR signals of DPPH and DPPH concentration were as follows: r=0.999 for absolute signal intensity, r=0.995 for absolute signal area, r=0.993 for relative signal intensity to MnO, and r=0.998 for relative signal area to MnO. The highest correlation coefficient value (r=0.999)

Table 1 ESR signals of DPPH in different solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Signal intensity</th>
<th>S/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS (10%DMSO)</td>
<td>199.4</td>
<td>90.6</td>
</tr>
<tr>
<td>DMSO</td>
<td>41.2</td>
<td>13.7</td>
</tr>
<tr>
<td>Ethanol</td>
<td>24.4</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Fig. 1 ESR spectra of DPPH radical dissolved in (a) PBS, (b) ethanol, or (c) DMSO. DPPH was dissolved in PBS(10%DMSO), ethanol or DMSO at the final concentration of 100μM, and ESR spectra were recorded at 23°C.
was obtained for absolute signal intensity, and therefore the absolute signal intensity was chosen to evaluate the DPPH concentration in the sample solution. The signal intensity of DPPH changed little by sodium selenite, sodium selenate, diphenyldiselenide or ebselen (Fig.2). Selenocystamine (SeCyst), selenomethionine (SeMet), DEDC-Na, and GSSG reduced the signal intensity by 10-20% (Fig.2). DEDC-SeT reduced the signal intensity by ~40% (Fig.2), which exceeded DEDC-SS or DEDC-Na indicating that selenium is involved in the activity of DEDC-SeT. The effect of DEDC-SeT was higher than that of ascorbic acid and was similar to that of GSH or DTT.

Although antioxidant mechanism of DEDC-SeT is not clear, it may be possible that selenium in DEDC-SeT is oxidized to Se-OH by hydroxyl radical catalytically produced from H₂O₂ and nitrogen radical in DPPH is reduced to =N-H by hydrogen radical, the residual part of H₂O₂.

The signal intensity of DPPH was slightly reduced by heparin, Hep-Cyst, or GSSG (Fig.3). The Hep-SeCyst, Pul-SeCyst, Lam-SeCyst, and Fuc-SeCyst at 100µM had reduced DPPH signal intensity by 10-30% (Fig.3). These conjugates had almost the same activity as that of ascorbic acid at the same concentration. In the case of Hep-SeCyst, heparin and Hep-Cyst reduced the signal intensity by less than 5% (Fig.3), and Hep-SeCyst had much higher activity indicating that selenium is an essential constituent of the Hep-SeCyst to exhibit the DPPH radical scavenging activity. As the chemical form of selenium in the Hep-SeCyst is chiefly selenol [1], selenol is thought to be an important functional group in these conjugates. It is also indicated that selenol groups in Hep-SeCyst are more efficient antioxidant than thiol groups in Hep-Cyst as shown previously [6].

These results suggested that selenium is essential in the DPPH scavenging activity of DEDC-SeT and polysaccharide-SeCyst conjugates.

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References


