Scavenging Activity of MGN-3 (Arabinoxylane from Rice Bran) with Natural Killer Cell Activity on Free Radicals

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Summary

MGN-3, which produces a remarkable increase in natural killer cell activity, showed a high scavenging rate on hypoxanthine-xanthine oxidase generated superoxide anion radicals, and on ferrous sulfate-hydrogen peroxide and UV light reaction system generated hydroxyl radicals. The S-group of MGN-3 fractions (L>10,000 molecular, 10,000>M>3,000 molecular and 3,000 molecular>S) showed the highest scavenging rate on superoxide anion radicals and the UV light reaction system. There was no difference in the scavenging rate for hydroxyl radicals by the Fenton reaction.

Key words: Biobran, Arabinoxylane, MGN-3, Free radical

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Introduction

MGN-3 (Biobran) is a modified arabinoxylan derived from rice bran, which consists of modified hemicellulose produced by treating the water-soluble hemicellulose fraction with enzymes (carbohydrases) from shiitake fungi culture filtrate. MGN-3 is an arabinoxylan with a xylose in its main chain and an arabinose polymer in the side chain (Figure 1). It has been reported to increase NK cell activity in cancer patients with decreased immunity\(^1\). This compound and its fractions, separated with a Sephadex G-25 column, were measured for their active-oxygen (\(\cdot\)O\(_2\)\(^-\)) scavenging activities using electron spin resonance (ESR: Japan Electronics JES-FR-30).

I Materials and Methods

Eight g of material components of MGN-3 was dissolved in 400 ml of ethanol and its supernatant was condensed by an evaporator. The subsequent component was eluted and fractionated by a Sephadex G-25 column, and each component was measured for their active-oxygen (\(\cdot\)O\(_2\)\(^-\), \(\cdot\)OH) scavenging activities using electron spin resonance (ESR: Japan Electronics JES-FR-30).

Active enzyme scavenging activity was evaluated by measuring the superoxide anion radical (\(\cdot\)O\(_2\)) scavenging activity, hydroxyradical (\(\cdot\)OH) scavenging activity of the Fenton reaction, and the scavenging activity of the hydroxyradical generated from ultraviolet irradiation. 

\(\cdot\)O\(_2\) scavenging activity was measured by the Spin Trap method using the HPX-XOD reaction. Namely, 50 µl of 2 mM hypoxanthine solution (HPX), 35 µl of 5.5 mM DETA-PAC solution, 15 µl of 9.2 M DMPO and 50 µl of sample solution were mixed, and timekeeping was started concurrently with the addition of 50 µl of 0.4 U/ml xanthine oxidase solution (XOD). After stirring for 1 min, the spectrum of \(\cdot\)O\(_2\) adduct generated inside the 120 min quartz cell was measured. The scavenging activity was calculated as a relative signal strength of \(\cdot\)O\(_2\) adduct to the signal strength of the internal standard Mn. It was also calculated as the SOD concentration corresponding to the SOD activity of the sample, from the calibration curve of SOD at various concentrations.

\(\cdot\)OH was measured using the Fenton reaction. Fifty ml of sample solution from each group was added to 75 µl of 1 mM FeSO\(_4\) and mixed with 20 µl of 9.2 M 10-fold diluted DMPO. Then, 75 µl of 0.1 mM H\(_2\)O\(_2\) was added, stirred for two minutes, placed in the cell, and sweeping was started 60 sec after H\(_2\)O\(_2\) was added.

\(\cdot\)OH was further measured using the generation system of the ultraviolet reaction. Two hundred and fifty ml of sample solution and 40 µl of 9.2 M 10-fold diluted DMPO were mixed, 150 µl of 100 mM H\(_2\)O\(_2\) was added, stirred, poured into a plastic container and after ultraviolet irradiation (365 nm, 4 \(\times\) 10\(^3\)/J/m\(^2\)/min) for 5 min, it was placed in a cell and measured.

Spectral analysis using ESR (Japan Electronics JES-FR30) was performed under the following conditions:

- Magnetic field sweep width: 335.6 mT
- Magnetic field modulation: 0.1 mT
- Gain: 125
- Sweep time: 2 min
- Response time: 0.1 sec
- Temperature: room temperature
II Results

The \( \cdot O_2 \) scavenging activity of MGN-3 was dose-dependant: 64.6%, 23.0%, and 4.4% as scavenging rates at 20, 2.0, and 0.2 mg/ml, respectively. The SOD activities of samples for the calibration curve were 7.6, 0.9, and 0 U/ml, respectively. The \( \cdot OH \) scavenging activity, as determined by the Fenton reaction, was also dose-dependant: scavenging rates were 94.9%, 78.9%, and 3.3% at 20, 2.0, and 0.2 mg/ml, respectively. The UV-generated \( \cdot OH \) scavenging activity was 72.6%, 35.9%, and 11.5% as scavenging rates (Table 1).

The \( \cdot O_2 \) scavenging activity of each fraction of MGN-3 increased with dose: scavenging rates were 39.9%, 10.4%, and 0% at 20, 2.0, and 0.2 mg/ml for the L fraction; 49.5%, 15.6%, and 0% for the M fraction; and 90.4%, 68.1%, and 26.4% for the S fraction. The S fraction had the highest scavenging activities. The SOD activities of the L, M, and S fractions were 5.0, 7.2, and 70.5 U/ml at 20 mg/ml; 0.8, 1.4, and 15.7 U/ml at 2.0 mg/ml; and 0, 0, and 2.6 U/ml at 0.2 mg/ml (Table 1).

The \( \cdot OH \) scavenging activities of each fraction of MGN-3 at 20, 2.0, and 0.2 mg/ml were 97.2%, 34.4%, and 3.3% for the L fraction; 97.0%, 68.4%, and 8.7% for the M fraction; and 96.5%, 55.1%, and 4.2% for the S fraction in terms of scavenging rate. All the fractions had high activities. The UV-generated \( \cdot OH \) scavenging activities at 20, 2.0, and 0.2 mg/ml were 41.8%, 16.5%, and 1.0% for the L fraction; 45.4%, 9.9%, and 3.9% for the M fraction; and 71.0%, 54.9%, and 19.6% for the S fraction in terms of scavenging rates. The S fraction had the highest scavenging activities (Table 1).

Conclusion

The active oxygen-scavenging activity of Biobran, a plant polysaccharide processed food, was investigated in this study. We found that Biobran had a high scavenging activity on \( \cdot O_2 \) and \( \cdot OH \) involved in ageing and diseases. The \( \cdot OH \) scavenging effect on the Fenton reaction was especially prominent.

The results of the measurements are shown in Table. The S fraction excelled all others in the inhibition of \( \cdot OH \) generation caused by \( \cdot O_2 \) and ultraviolet irradiation, and high scavenging activity was observed in all fractions for the scavenging activity of \( \cdot OH \) generation in the Fenton reaction).
<table>
<thead>
<tr>
<th>Kind of Active Oxygen and SOD activity</th>
<th>Scavenging ratio of Superoxyde anion radical (%)</th>
<th>SOD activity (U/ml)</th>
<th>Scavenging ratio of Hydroxyl radical by UV light reaction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 □mg/ml □</td>
<td>0.2</td>
<td>20 □mg/ml □</td>
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<tr>
<td>MGN-3</td>
<td>64.6 □20 □</td>
<td>4.4</td>
<td>7.6 □2.0 □</td>
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<td></td>
<td>78.9 □2.6 □</td>
<td>3.3</td>
<td>94.9 □5.9 □</td>
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<tr>
<td>MGN-3-L</td>
<td>39.9 □20 □</td>
<td>0</td>
<td>5.0 □2.0 □</td>
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<tr>
<td></td>
<td>34.4 □6.5 □</td>
<td>3.3</td>
<td>97.2 □6.5 □</td>
</tr>
<tr>
<td>MGN-3-M</td>
<td>49.5 □20 □</td>
<td>0</td>
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<tr>
<td></td>
<td>68.4 □6.5 □</td>
<td>8.7</td>
<td>97.0 □6.5 □</td>
</tr>
<tr>
<td>MGN-3-S</td>
<td>90.4 □20 □</td>
<td>26.4</td>
<td>70.5 □2.0 □</td>
</tr>
<tr>
<td></td>
<td>55.1 □4.9 □</td>
<td>4.2</td>
<td>96.5 □4.9 □</td>
</tr>
</tbody>
</table>

·O₂: HPX-XOD reaction, ·OH: Fenton reaction, 
·OH by UV light reaction: 365 nm, 4 × 10⁻¹¹J/m²/min × 5

Reference