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 ($\cdot O_2^-$, $\cdot OH$) 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 named in descending order as L (L > 10,000 molecule), M (10,000 > M > 3,000 molecule), and S (3,000 molecule > S), and used for the evaluation. Ultrapure water was added to these material components to make 20 mg/ml, subjected to shaking extraction for 10 minutes, and centrifuged (3,000 rpm, 5 min) to obtain the supernatant. The supernatant was further diluted to make 2.0 mg/ml and 0.2 mg/ml sample solutions, and used for measurement.

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.

·O2 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.

·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_2O_2 was added, stirred for two minutes, placed in the cell, and sweeping was started 60 sec after H_2O_2 was added.

·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_2O_2 was added, stirred, poured into a plastic container and after ultraviolet irradiation (365 nm, 4×10^3/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

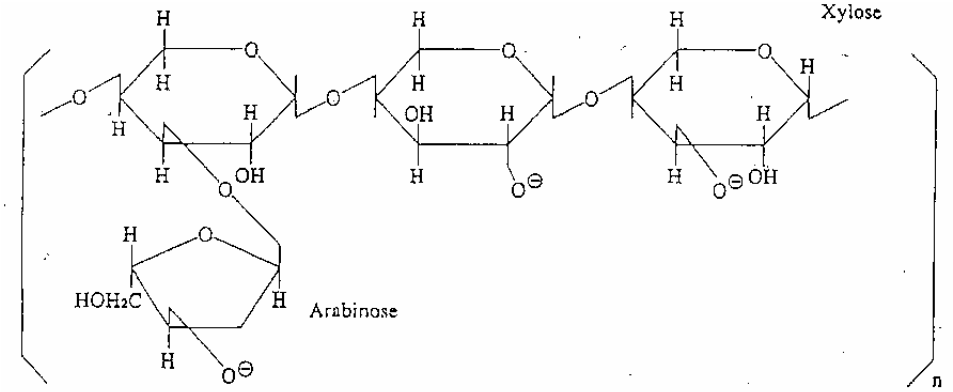

Fig. 1 Arabino xylane model in bio bran

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 ·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 ·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 ·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 ·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 O_2^- and OH involved in ageing and diseases. The 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 ·OH generation caused by $\cdot O_2^-$ and ultraviolet irradiation, and high scavenging activity was observed in all fractions for the scavenging activity of ·OH generation in the Fenton reaction).

Table 1

Scavenging Activity of MGN-3 on Active Oxygen radical ($\cdot O_2^-$ and $\cdot$OH and UV light reaction $\cdot$OH)

Kind of Activie Oxygen and SOD activity	Scavenging ratio of Superoxyde anion radical (%)			SOD activity (U/ml)			Scavenging ratio of Hydroxyl radical by UV light reaction (%)		
	20	2.0 (mg/ml)	0.2	20	2.0 (mg/ml)	0.2	20	2.0 (mg/ml)	0.2
MGN-3	64.6	23.0	4.4	7.6	0.9	0	94.9 (72.6)	78.9 (35.9)	3.3 (11.5)
MGN-3-L	39.9	10.4	0	5.0	0.8	0	97.2 (41.8)	34.4 (16.5)	3.3 (1.0)
MGN-3-M	49.5	15.6	0	7.2	1.4	0	97.0 (45.4)	68.4 (9.9)	8.7 (3.9)
MGN-3-S	90.4	68.1	26.4	70.5	15.7	2.6	96.5 (71.0)	55.1 (54.9)	4.2 (19.6)

$\cdot O_2^-$: HPX-XOD reaction, $\cdot$OH: Fenton reaction,

$\cdot$OH by UV light reaction: 365 nm, $4 \times 10^3 J/m^2/min \times 5$

Reference

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