Note

Active Oxygen Scavenging Activity of DDMP (2,3-Dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) Saponin in Soybean Seed

Yumiko Yoshiki and Kazuyoshi Okubo

Department of Applied Biological Chemistry, Faculty of Agriculture, Tohoku University, Tsutsumidori, 1-1 Amamiya-machi, Sendai 981, Japan

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DDMP saponin, which is widely distributed in leguminous seeds, scavenged oxygen radicals when assayed using the xanthine oxidase-NH₂OH method, electron spin resonance (ESR), and chemiluminescence. One mg of DDMP saponin/ml scavenged superoxide at a degree equivalent to 17.1 units of superoxide dismutase/ml by the ESR spin trapping method. This scavenging activity of DDMP saponin is caused by the DDMP moiety attached to the triterpene aglycone since soybean saponin Bb, which is derived from soyasaponin βg, but which lacks this group, did not show the scavenging activity.

We have isolated genuine saponins, soyasaponin αg, βg, βa, γg, and γa from soybean1 and soyasaponin αa from scarlet runner bean2 and have shown them to contain an unusual sugar, 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) attached through an ether linkage to the C-22 hydroxyl group of the soyasapogenol B. Since DDMP saponins are widely distributed in the hypocotyl of leguminous seeds, these saponins were considered to have some important physiological activity. In this paper, the radical scavenging activity of DDMP saponin was measured against superoxide radicals (O₂⁻) using the enzymatic xanthine oxidase-NH₂OH method, electron spin resonance (ESR) spectrometry, and chemiluminescence (CL) in the hypoxanthine (HX) and xanthine oxidase (XOD) systems, respectively. The scavenging activity was measured on other active oxygen, such as hydrogen peroxide (H₂O₂), hydroxyl radical (HO·), and lipid hydroperoxide (LOOH), using CL.

DDMP saponin was isolated from hypocotyls of soybean as described previously.3) Soybean saponin Bb was obtained from soyasaponin βg following hydrolysis at 100°C for 2 h.

O₂⁻ scavenging activity of DDMP saponins and related substances was measured by the XOD-NH₂OH enzymatic method and compared with that of glutathione (Sigma Chem.) which was known to relate to age4) and oxidant stress.5) The activity was measured in about 100 μl of each concentration, 5-15 mM, according to McCord and Fridovich,6) and Shinohara.7) O₂⁻ scavenging activity was indicated from the scavenging ratio (100(ES-EB/EC-EB)×100, EB, blank, EC, control, ES, sample) by absorption spectrophotometry at 550 nm. Fifteen mM of glutathione showed a strong (70.7%) scavenging activity of O₂⁻. Although soybean saponin Bb had no activity (0%), soyasaponin βg showed the activity of 5 mM concentration (8.5%). Maltol, which was thought to be a derivative from the DDMP moiety of DDMP saponin, also showed a scavenging activity from 5 mM. This suggested that the O₂⁻ scavenging activity of DDMP saponin was caused by the DDMP moiety. The scavenging activity of 15 mM soyasaponin βg was 42.7%, which corresponded to 2/3 of that of glutathione and twice that of maltol.

In the ESR spin trapping method, 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) was used to bind the O₂⁻, which was generated by the HX and XOD system (DMPO-O₂⁻). The O₂⁻ scavenging activity was calculated using the index of the DMPO-O₂⁻ spin adduct by comparison with that of bovine erythrocyte superoxide dismutase (SOD), which was purchased from Sigma Chem., as described by Mitsuta et al.8) O₂⁻ scavenging activity of 1 mg of soyasaponin βg/ml measured by the ESR method, was found to be equivalent to 17.1 units of SOD/ml (Fig.).

The CL of DDMP saponin was measured in the presence of O₂⁻. The instrument used was a filter-equipped photon counter-type spectrometer (CLD-110, Tohoku Electronic Ind.), connected to a Waters Model 510 pump and U6K injector. O₂⁻ was generated by addition of 5 mM HX (10 μl) and 100 mg/ml XOD (1 μl) to a mobile solvent, 50 mM phosphate buffer containing 2% CH₃CHO (pH 7.0); the flow rate of 1 ml min⁻¹ at 23°C was different to that used by Watanabe et al.9) The dispersed light at the grating was simultaneously detected on the photocathode with the image sensor set at wavelengths from 300 to 650 nm. The photons counted at the respective wavelengths were computed as spectral intensities. The CL intensity (12.5 photon counts s⁻¹, attn. 8) of 5 mM glutathione (100 μl) in the presence of O₂⁻ by the HX-XOD system, and CH₃CHO was arbitrarily deferred as 1.00 unit to allow comparison of the CL intensity of DDMP saponins and maltol (Table). The CL of soyasaponin αg, which was a different sugar moiety (Glc-Gal-GluA), from soyasaponin βg(Rham-Gal-GluA) was slightly reduced in intensity compared with the latter. This suggested CL with O₂⁻ and DDMP saponin

Abbreviations: DDMP, 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one; O₂⁻, superoxide anion; CL, chemiluminescence; ESR, electron spin resonance; HX, hypoxanthine; XOD, xanthine oxidase; DMPO, 5,5-dimethyl-1-pyrroline-1-oxide; SOD, superoxide dismutase; HO·, hydroxyl radical; H₂O₂, hydrogen peroxide; LOOH, lipid hydroperoxide.
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When scavenging activity against lipid hydroperoxide (LOOH) was measured by using 50 μM phosphate buffer containing 2% tert-buty1 hydroperoxide, which is the shortest chain hydroperoxide, and 2% CH3CHO as a mobile solvent, according to the method described above, DDMP saponin did not show LOOH scavenging activity.

In the organism, oxygen radicals have been implicated in the initiation and development of various diseases, often mediated through chain reactions of lipid peroxidation and DNA- and protein-binding and degeneration.10,11) Our results suggest that DDMP saponins have the possibility to prevent biomolecular damage due to radical attack.

Although the principle and radical species for the formation the CL have not been identified, the phenomenon of bioluminescence has been observed in luciferase12) and soybean seedlings,8) suggesting that the CL may occur in the same mechanism as that of bioluminescence. As compared with the ESR spin trapping technique and enzymatic methods, a direct measurement of the CL in the presence of aldehyde and oxygen radical is such a new method to search for radical scavengers.

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References