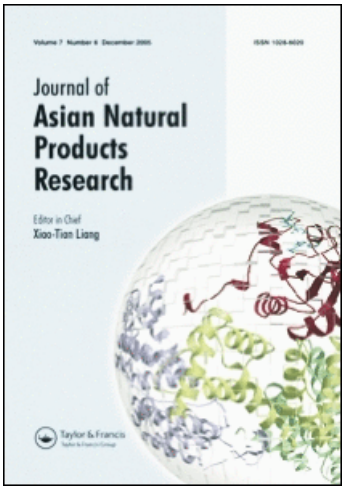

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An *In Vitro* Study of the Structure-Activity Relationships of Sulfated Polysaccharide from Brown Algae to its Antioxidant Effect

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AN *IN VITRO* STUDY OF THE STRUCTURE-ACTIVITY RELATIONSHIPS OF SULFATED POLYSACCHARIDE FROM BROWN ALGAE TO ITS ANTIOXIDANT EFFECT

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In this paper, the structure-activity relationships of chemically modified uronic acid polymer fragments from brown algae with regard to their antioxidant effects on H_2O_2-damaged lymphocyte were studied. The results indicated that the most potent antioxidant activity was obtained from the sulfated polysaccharide with ratio of mannuronate blocks (M-blocks) to guluronate blocks (G-blocks) of 3 to 1 and carboxyl residue unesterified. The sulfated G-blocks with esterified carboxyl residue also prevented lymphocyte from injury. However, the sulfated G-blocks bearing unesterified carboxyl residue hardly exerted antioxidant activity. These findings suggested that both M-blocks and esterified carboxyl residue were determinant structures in preventing lymphocyte from being oxidized by H_2O_2, indicating that the existence of M-blocks was more important in scavenging free radicals.

Keywords: HSH91; HSH92; HSH87; Lymphocyte; Antioxidant activity; H_2O_2; Kunming mice

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INTRODUCTION

A free radical is any species capable of independent existence that contains one or more unpaired electrons [1]. An enormous literature has shown that free radicals attack double bounds in polyunsaturated fatty acids and other targets, which may result in changes of the general architecture and permeability properties of cell membrane, the breakdown of chain of protein and nucleic acid, the necrosis and apoptosis of cells [2]. Oxygen radical is involved in many physiological and pathological processes such as stimulation of HIV replication and the development of immunodeficiency [3, 4]. Reactive oxygen may also play a critical role in cardiovascular diseases such as atherosclerosis that has also been reviewed due to its impairment of endothelial cell [5]. Therefore, the reversal or blockade of any processes involved in oxidative injury might provide promising features of antioxidants.

Polysaccharides are very important biological species in many life processes. Because of their structural diversity and multilaterally functional importance, the research and development have already drawn much attention recently. Sulfated polysaccharides such as glycosaminoglycans (GAGs) and trehalose have been demonstrated to play a pivotal role in the pathogenesis of basement membrane-related diseases, including atherosclerosis, HIV infection, diabetes, tumor metastasis and so on [6–9]. But the underlying mechanisms of their actions are not fully understood.

HSH91, HSH92 and HSH87 are all sulfated polysaccharides extracted from brown algae by enzymatic depolymerization, following chemical modification. Studies on the structure-activity relationships of marine sulfated polysaccharides with regard to their antioxidant effects on H_2O_2-induced lymphocyte were investigated here. The chemically modified sulfated polysaccharides were tested for their ability to protect lymphocyte from injury *in vitro*, in order to elucidate their structural requirements of the antioxidant activity.

RESULTS AND DISCUSSION

Protective Effect of HSH91 on Lymphocyte Injury Induced by H_2O_2

Exposure of lymphocyte to H_2O_2 at concentration of 25 μM resulted in marked cell injury characterized by cell loss, suggestive of successful establishment of damage model. HSH91 at concentrations of 0.001, 0.01,

0.1, 1, 10 $\mu g \cdot ml^{-1}$ dose-dependently increased cell counts of lymphocyte as compared with those of model group. The maximal antioxidant activity was observed at 10 $\mu g \cdot ml^{-1}$ with antioxidant rate equivalent to 36.8% ($p < 0.001$) (shown in Tab. I).

Protective Effect of HSH87 on Lymphocyte Injury Induced by H_2O_2

Effect of HSH87 on lymphocyte damage was also evaluated by determination of cell viability. HSH87 at given concentrations significantly enhanced the viability of lymphocyte, and HSH87 at dose of 10 $\mu g \cdot ml^{-1}$ showed the maximal antioxidant effect ($p < 0.001$) (shown in Tab. II).

Protective Effect of HSH92 on Lymphocyte Injury Induced by H_2O_2

Concomitant incubation of lymphocyte with H_2O_2 for 2h led to oxidative injury. In HSH92-treated groups, the viable cell counts were not

TABLE I Protective effect of HSH91 on lymphocyte injury induced by H_2O_2

Group	*Concentration* ($\mu g \cdot ml^{-1}$)	*Absorbance*	*Antioxidant rate* (%)
Normal	–	0.471 ± 0.017	
Control	–	0.325 ± 0.017###	–
HSH91	0.001	0.361 ± 0.027*	11.1
	0.01	0.378 ± 0.035**	16.4
	0.1	0.405 ± 0.015***	24.7
	1	0.413 ± 0.045***	27.0
	10	0.445 ± 0.036***	36.8

$\bar{x} \pm SD(n = 6)$; ### $P < 0.001$ *vs.* normal group, * $p < 0.05$, ** $p < 0.01$, *** $P < 0.001$ *vs.* control group.

TABLE II Protective effect of HSH87 on lymphocyte injury induced by H_2O_2

Group	*Concentration* ($\mu g \cdot ml^{-1}$)	*Absorbance*	*Antioxidant rate* (%)
Normal	–	0.560 ± 0.023	–
Control	–	0.404 ± 0.023###	–
HSH87	0.001	0.466 ± 0.033**	15.3
	0.01	0.460 ± 0.012***	13.9
	0.1	0.441 ± 0.013**	9.2
	1	0.500 ± 0.025***	23.7
	10	0.523 ± 0.017***	29.8

$\bar{x} \pm SD(n = 6)$; ### $P < 0.001$ *vs.* normal group, ** $P < 0.01$, *** $P < 0.001$ *vs.* control group.

TABLE III Protective effect of HSH92 on lymphocyte injury induced by H_2O_2

Group	*Concentration* ($\mu g \cdot ml^{-1}$)	*Absorbance*	*Antioxidant rate* (%)
Normal	–	0.545 ± 0.018	
Control	–	$0.436 \pm 0.023^{\#\#\#}$	–
HSH92	0.001	0.451 ± 0.019	–
	0.01	0.441 ± 0.025	
	0.1	0.429 ± 0.028	–
	1	$0.445 + 0.022$	–
	10	0.473 ± 0.018**	8.5

$\bar{x} \pm SD(n = 6)$; $^{\#\#\#}$ $P < 0.001$ *vs.* normal group, ** $P < 0.01$ *vs.* control group.

statistically different from those of model with exception of HSH92 at dose of $10\,\mu g \cdot ml^{-1}$. The antioxidant potency of HSH92 was significantly lower than that of HSH91 or HSH87.

Increasing evidence has indicated that biological diversities of polysaccharides not only reside in their structural heterogeneity, molecular weights and sequence alignments, but also depend on the molecular modification including the pattern, number and position of substitution of residues [10]. Many observations have indicated that sulfation pattern and degree lead to differences in bioactivity [11, 12]. Previous works from our laboratory had demonstrated distinct anti-HIV and anticoagulant activities resulting from different sulfation degrees of polysaccharides. Our present findings indicated that the compounds with the same pattern and degree of sulfation but different sequence alignments of polysaccharide exerted different antioxidant effects, implying that sulfated modification might not be the unique determinant of antioxidant activities.

In fact, it is acknowledged that sequence variation results in biological diversities. It highlighted the importance of the ratio of mannuronate blocks over guluronate blocks (M/G) in biological functions as evidenced by our previous work [10]. Our results further illustrated better antioxidant potency of carbohydrate backbone bearing M/G ratio equivalent to 3 to 1 than that of pure guluronate blocks as HSH92 and HSH87. These gave us a good explanation that the co-existence of both mannuronate and guluronate blocks might provide an appropriate conformation to counteract the oxidative damage.

Furthermore, regardless of presence of the same sulfation pattern with the same sequence composition, sulfated polysaccharide with esterified carboxyl groups as HSH87 showed better antioxidant potency than that with unesterified carboxyl residue as HSH92, suggestive of the paramount role of

esterified carboxyl residue in scavenging free radicals. These were in line with the notions that the different substitution of residues resulted in the structural and subsequently conformational change, which led to the variation in biological functions.

In addition, as demonstrated by a large body of evidence that biological diversity of sulfated polysaccharides not only benefited from their sequence alignment but also from the pattern of molecular modification [10]. Our results indicated that HSH91 with unesterified carboxyl was a potent scavenger of free radical compared to HSH87 with esterified carboxyl residue. This raised the possibility that the co-existence of both mannuronate and guluronate blocks at unique ratio was much more important than esterified carboxyl residue in maintaining desired structural conformation to scavenge free radicals.

In conclusion, this is the first time to report that the ratio of M/G and the presence of esterified carboxyl residue determine the potency of marine sulfated polysaccharides derived from brown algae in scavenging free radical.

EXPERIMENTAL SECTION

Preparation of Sulfated Polysaccharides

HSH91, HSH92 and HSH87, at average molecular weights of approximately 6000Da, were sulfated polysaccharides extracted from marine brown algae *Ecklonia Kurome Okam* and depolymerized by enzymatic digestion, following sulfated modification. HSH91 bore 3/1 ratio of 1,4-linked β-D-mannuronic acid blocks to α-L- guluronic acid blocks. And HSH92 bore pure α-L- guluronic acid blocks with unesterified carboxyl group. While HSH87 had the same sequence alignment as HSH92 but with the carboxyl residue esterified.

Lymphocyte Culture

Lymphocyte was isolated from thymus of Kunming mice, weighing 20 ± 0.5 g. Cell culture was modified as follows [7]. Briefly, the mice were killed by cervical dissection. The thymus was excised and cleaned of blood, and then was ground on 60–100 order metal mesh. The suspension was centrifuged at 1000 rev./min for 5 min, and the lymphocyte was counted by staining with 3% trypan blue.

The cells for each experiment were plated with an initial cell density at 2×10^6cells/well into 96-well plates and incubated at 37°C.

Evaluation of H_2O_2-induced Oxidative Stress on Lymphocyte

The lymphocyte of 2×10^6cells/well were seeded into 96-well plates. Exposure of HSH91, HSH92 and HSH87 at the final concentrations ranging from 0.001 to 10 $\mu g \cdot ml^{-1}$ to lymphocyte was concomitantly incubated with H_2O_2 at concentration of 25 μM except for the control group. Two hours later, the antioxidant activities of HSH91, HSH92 and HSH87 were evaluated by MTT assay. The percentage of antioxidant activity was calculated according to the following formula: antioxidant rate (%) = (absorbance of drug-treated cells/H_2O_2-treated cells-1) × 100.

Statistical Analysis

Data were expressed as $\bar{x} \pm SD$ and analyzed by the Students' t-test.

References

[1] Halliwell, B. (1993). *Haemostasis*, **23**(suppll), 118–126.
[2] Hu, J. F., Geng, M. Y., Yu, G. L. and Xu, J. M. (2000). *Chinese journal of Marine drugs*, in press.
[3] Allard, J. P., Aghdassi, E., Chau, J., Sali, I. and Walmsley, S. (1988). *Am. J. Clin Nutr.*, **67**, 143–147.
[4] Schrech, R., Rieber, P. and Baeuerle, P. A. (1991). *EMBO J.*, **10**, 2247–2258.
[5] Engelberg, H. (1996). *Pharmacological Reviews*, **48**(2), 327–351.
[6] Jackson, R. L., Busch, S. J. and Cardin, A. D. (1991). *Physiological reviews*, **71**(2), 481–539.
[7] Zhang, J. T. (1998). *Experimental methods of modern pharmacology*, pp. 649–650.
[8] Tian, X. H., Cong, J. B., Shi, D. J., Sun, C. P. and Gu, J. F. (1997). *Acta Nutrimenta Sinica*, **19**(1), 32–37.
[9] Itoh, H., Noda, H. and Amano, H. (1993). *Anticancer Res.*, **13**, 2045–2054.
[10] Geng, M. Y., Xin, X. L., Li, Y. X. and Guan, H. S. (2000). *Chinese journal of Marine drugs*, 2000, in press.
[11] Castellot, J. J., Beeler, D., Rosenberg, R. D. and Karnovsky, M. (1984). *J. Cellular Physiology*, **120**, 315–319.
[12] Hari, G., Joseph, A. M., Thompson, B. T., Hales, C. A., Toida, T., Imanari, T., Capila, I. and Linhardt, R. J. (1999). *Article of Biochemistry and Biophysics*, **371**(2), 228–235.