Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 346 (2006) 345–350
BBRC
www.elsevier.com/locate/ybbrc

Bifunctional effects of fucoidan on the expression of inducible nitric oxide synthase

Jin Won Yang [a], Se Young Yoon [a,b], Soo Jin Oh [c], Sang Kyum Kim [c], Keon Wook Kang [a,*]

[a] College of Pharmacy, Chosun University, Gwangju, South Korea
[b] LG Household and Healthcare Ltd., Research Park, Daejeon, South Korea
[c] College of Pharmacy and Research Center for Transgenic Cloned Pigs, Chungnam National University, Daejeon, South Korea

Received 19 May 2006
Available online 2 June 2006

Abstract

Algal fucoidan is a marine sulfated polysaccharide with a wide variety of biological activities including anti-thrombotic and anti-inflammatory effects. This study evaluated the effect of fucoidan on the expression of inducible nitric oxide synthase (iNOS) in a macrophage cell line, RAW264.7. Low concentration range of fucoidan (10 μg/ml) increased the basal expression level of iNOS in quiescent macrophages. However, we found for the first time that fucoidan inhibited the release of nitric oxide (NO) in RAW264.7 cells stimulated with lipopolysaccharide (LPS). Western blot analysis revealed that fucoidan suppressed the LPS-induced expression of the inducible nitric oxide synthase (*iNOS*) gene. Moreover, the activation of both nuclear factor-κB (NF-κB) and activator protein 1 (AP-1) are key steps in the transcriptional activation of the *iNOS* gene. Here, it was revealed that fucoidan selectively suppressed AP-1 activation, and that the activation of AP-1 appears to be essential for the induction of iNOS in activated macrophages. This inhibitory effect on AP-1 activation by fucoidan might be associated with its NO blocking and anti-inflammatory effects.

Keywords: AP-1; Fucoidan; iNOS; Macrophages; Nitric oxide

Algal fucoidan, which is extracted from brown algae (e.g., Ascophyllumnodosum and Fucus vesiculosus), is a marine sulfated polysaccharide that is endowed with important biological activities including anti-viral, anti-angiogenic, anti-tumoral, contraceptive, anti-thrombotic, anti-coagulant, and anti-inflammatory effects [1]. The brown seaweed, *Laminaria japonica* Aresch. (Laminariales), is distributed widely in East Asia and is consumed as a marine vegetable. Fucoidan, one of its main constituents, is also available as food supplement in Japan and United States.

Fucoidan contains a substantial percentage of L-fucose and sulfate ester groups, and the structural characteristics of fucoidan are very similar to those of heparin. Therefore, it exhibits potent anti-thrombotic activity [1]. Another outstanding physiological function of fucoidan is anti-inflammatory properties through anti-complementary [2], anti-leukocyte migration [3] and anti-proliferation effects on smooth muscle cell [4]. In addition, fucoidan as a ligand for the macrophage scavenging receptor 1 (MSR1) increased the level of tumor necrosis factor-α (TNF-α) and interleukin-1 secretion [5]. It was recently reported that fucoidan increases the level of nitric oxide (NO) production in quiescent macrophages, which was related with p38 kinase-dependent NF-κB activation [6].

Although several studies on the biological activities of fucoidans have been performed, with particular focus on its immunomodulatory actions, it is unclear if fucoidan affects the formation of NO in lipopolysaccharide (LPS; endotoxin, a representative Toll-like receptor 4 agonist)-stimulated macrophages. This study we unexpectedly

* Corresponding author. Fax: +82 62 222 5414.
E-mail address: kwkang@chosun.ac.kr (K.W. Kang).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.05.135

found that fucoidan inhibits the LPS-mediated expression of inducible nitric oxide synthase (iNOS) by blocking AP-1 activation in macrophages.

Materials and methods

Materials. The 5-bromo-4-chloro-3-indoylphosphate and nitroblue tetrazolium solutions were purchased from Promega (Madison, WI); the anti-murine iNOS polyclonal antibody from Transduction Laboratories (Lexington, KY); the anti-phospho-I-κB antibody from Cell Signaling Technology (Beverly, MA), and the anti-c-Rel (p65) and I-κBα antibodies from Santa Cruz Biotechnology (Santa Cruz, CA). The horseradish peroxidase-conjugated donkey anti-rabbit and alkaline phosphatase-conjugated donkey anti-mouse IgGs were obtained from Jackson Immunoresearch Laboratories (West Grove, PA). All the reagents including fucoidan were supplied by Sigma (St. Louis, MO).

Cell culture. The RAW264.7 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and incubated at 37 °C in a 5% CO_2/95% air atmosphere in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS), 50 U/ml penicillin, and 50 μg/ml streptomycin. For all experiments, the cells were grown until they reached 80% to 90% confluence and were subjected to no more than 20 cell passages.

Measurement of NO. The RAW264.7 cells (5×10^5 cells) were preincubated in serum-free medium at 37 °C for 12 h and the level of NO production was monitored by measuring the nitrite levels in the culture media using the Griess reagent [7]. The absorbance was measured at 540 nm after incubating the culture medium with the Griess reagent for 10 min.

Preparation of nuclear extract. The cells were preincubated for 10 min in the culture medium in the presence or absence of sumaflavone or robustaflavone, and then exposed to LPS (1 μg/ml). The cells were then removed using a cell scraper, centrifuged at 2500*g* at 4 °C for 5 min, and swollen by adding 100 μl of a lysis buffer (10 mM Hepes (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.5% Nonidet P40, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonylfluoride). The cells were vortexed in order to disrupt the cell membranes, and the samples were incubated for 10 min on ice followed by centrifugation for 5 min at 4 °C. Pellets containing the crude nuclei were resuspended in 60 μl of the extraction buffer containing 20 mM Hepes (pH 7.9), 400 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonylfluoride, and incubated for 30 min on ice. The samples were then centrifuged at 15,800*g* for 10 min to obtain a supernatant containing nuclear extracts, which were stored at −80 °C until needed.

Western blot analysis. Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and immunoblot analyses were performed, as described previously [7]. The cells were lysed in a buffer containing 20 mM Tris–Cl (pH 7.5), 1% Triton X-100, 137 mM sodium chloride, 10% glycerol, 2 mM EDTA, 1 mM sodium orthovanadate, 25 mM β-glycerophosphate, 2 mM sodium pyrophosphate, 1 mM phenylmethylsulfonylfluoride, and 1 μg/ml leupeptin. The lysates were centrifuged at 12,000*g* for 10 min to the remove debris, fractionated by 10% gel electrophoresis, electrophoretically transferred to nitrocellulose paper, and incubated with the primary antibodies and then with the alkaline phosphatase- or horseradish peroxidase-conjugated secondary antibodies. Finally, the papers were developed using either 5-bromo-4-chloro-3-indoylphosphate and nitroblue tetrazolium or an ECL chemiluminescence detection kit [8].

Construction of an iNOS promoter-luciferase construct and NF-κB reporter gene assays. The pGL-miNOS-1588 luciferase reporter assay system was used to determine the transcriptional activity of the *iNOS* gene. In order to generate the miNOS promoter-luciferase construct (pGL-miNOS-1588), the miNOS promoter region from −1588 to +165 bp was amplified by a polymerase chain reaction (PCR) and ligated into the pGEM-T easy vector (Promega, Madison, WI). The amplified product was subcloned into the *Kpn*I/*Bgl*II site of the pGL3-basic plasmid [9].

The cells were plated at a density of 3×10^5 cells/well in a 12-well plate and transfected the following day. A dual-luciferase reporter assay system (Promega, Madison, WI) was used to determine the promoteractivity. Briefly, the cells were transiently transfected with 1 μg of pGL-miNOS1588, pNF-κB-Luciferase, or pAP-1-Luciferase plasmid and 4 ng of the pRL-SV plasmid (Promega, Madison, WI) using the Genejuice® Reagent (Novagen, Madison, WI) and then exposed to LPS for 18 h. The firefly and *Renilla* luciferase activities in the cell lysates were measured using a luminometer (Turner Designs; TD-20, CA). The relative luciferase activities were calculated by normalizing the iNOS, NF-κB, or AP-1 promoter-driven firefly luciferase activities versus that of *Renilla* luciferase.

Gel shift assay. The double stranded DNA probe for the consensus sequence of AP-1 (1.75 pmol/μl, 5′-CGCTTGATGAGTCAGCCGGAA-3′) was purchased from Promega (Madison, WI) and used for the gel shift analysis after end-labeling the probe with [γ-^{32}P]ATP and T_4 polynucleotide kinase. The reaction mixture contained 2 μl of 5× a binding buffer containing 20% glycerol, 5 mM $MgCl_2$, 250 mM NaCl, 2.5 mM EDTA, 2.5 mM dithiothreitol, 0.25 mg/ml poly(dI–dC), 50 mM Tris–Cl (pH 7.5), 10 μg of the nuclear extracts, and sterile water to a total volume of 10 μl. After a 10 min preincubation period, a 1 μl probe (10^6 cpm) was added and the mixture was incubated at room temperature for 20 min. The specificity of DNA/protein binding was determined by a competition reaction using a 20-fold molar excess of the unlabeled AP-1 oligonucleotide. The samples were loaded onto 5% polyacrylamide gels at 100 V. The gels were then removed, dried, and autoradiographed.

Scanning densitometry and statistics. Scanning densitometry was carried out using FLA-7000 Image Scan & Analysis System (Fujifilm, Japan), and a paired Student's *t* test was used to examine the significant intergroup differences. Statistical significance was accepted at either $p < 0.05$ or $p < 0.01$.

Results

Induction of iNOS by low concentration range of fucoidan in quiescent macrophages

Fucoidan was reported to upregulate iNOS expression in quiescent macrophages [6]. The present study also confirmed that 10 μg/ml fucoidan slightly, but significantly increased the levels of the iNOS protein in the un-stimulated RAW264.7 cells (Fig. 1). However, the enhanced expression of iNOS disappeared in cell lysates obtained from macrophages treated with 30 or 100 μg/ml fucoidan. Hence, 10 μg/ml of fucoidan may act as a weak inducer of the iNOS gene in quiescent macrophages as a ligand of MSR but fucoidan at concentration above 30 μg/ml may have another function to repress the induction of iNOS.

Inhibitory effects of fucoidan on the induction of iNOS in activated macrophages

Quiescent macrophages can be activated by exposure to proinflammatory cytokines or LPS. When LPS (1 μg/ml) was added to the RAW264.7 cells, the level of NO production increased from 12 h (8.5-fold) and peaked at 48 h (13.2-fold) (Fig. 2A). Fucoidan significantly inhibited this increase in NO production in a concentration-dependent manner, with 100 μM fucoidan completely blocking the LPS-inducible NO production at 24 h (Fig. 2A).

It is unclear if the inhibition of NO formation by fucoidan is the result of the inhibition of iNOS gene expression. Therefore, the inhibitory effects of the different fucoidan concentrations on iNOS protein expression induced by LPS (1 μg/ml) were assessed. As shown in Fig. 2B,

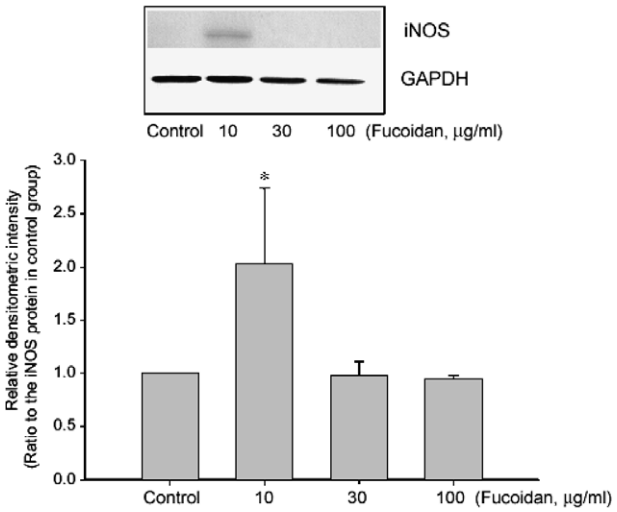

Fig. 1. Effect of fucoidan on iNOS expression in quiescent macrophages. RAW264.7 cells were incubated in a medium containing fucoidan (10–100 μg/ml) for 18 h and iNOS protein levels were monitored in the cell lysates. Relative iNOS protein levels were determined by measuring immunoblot band intensities by scanning densitometry. Data represent means ± SD of three separate experiments (significant compared to the untreated control, $*p < 0.05$).

fucoidan concentration-dependently inhibited iNOS protein expression at 30–300 μM, and iNOS induction by LPS was completely blocked by fucoidan concentrations >100 μM (Fig. 2B). The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) levels were comparable among samples. These results show that fucoidan suppresses the de novo synthesis of iNOS in LPS-activated macrophages.

iNOS expression is mainly regulated at the transcriptional level [10]. In order to determine if the process of *iNOS* gene transcription is targeted by fucoidan, reporter gene analysis was carried out using macrophages transfected with the mammalian cell expression vector, pGL-miNOS1588, which contained luciferase cDNA and a −1.59 kb miNOS promoter [9]. LPS (1 μg/ml) increased the luciferase activity by approximately 4.2-fold, which was reversed by 30 or 100 μM fucoidan (Fig. 2C).

No effect of fucoidan on LPS-inducible NF-κB activation

NF-κB is an essential transcription factor for the induction of several inflammatory mediators including, tumor necrosis factor-α, cyclooxygenase-2, and iNOS [12,13]. Therefore, a reporter gene assay was carried out using a luciferase plasmid containing the NF-κB minimal promoter to determine if the inhibition of iNOS induction by fucoidan is due to the suppression of NF-κB activation. The LPS treatment (18 h) caused a 2.9-fold increase in NF-κB reporter activity (Fig. 3A). However, a pretreatment with 30 or100 μM fucoidan had no effect on the LPS-induced luciferase activity (Fig. 3A).

The inhibitory protein I-κBα sequesters the activated NF-κB (a p65/p50 heterodimer) in the cytoplasm as an inactive complex. Upon inflammatory stimulation, its

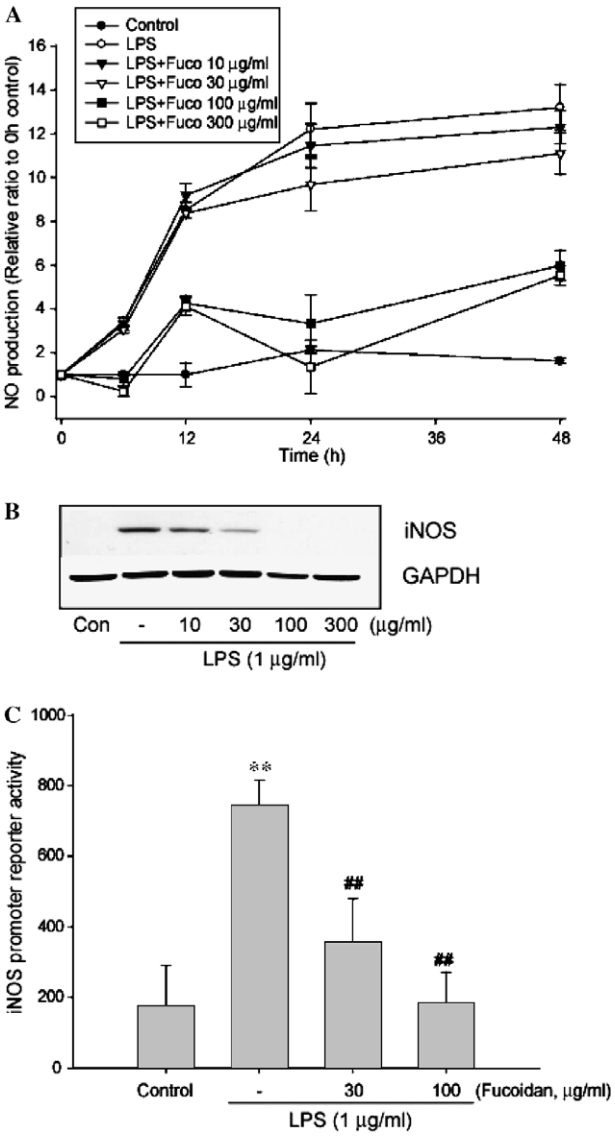

Fig. 2. Inhibition of iNOS induction by fucoidan in activated macrophages. (A) Effect of fucoidan on NO production in LPS-stimulated macrophages. RAW264.7 cells were incubated in a medium containing fucoidan (10–300 μg/ml) for 10 min and then treated with LPS at 1 μg/ml. The amount of nitrite generated in medium was monitored for 48 h. Data represent means ± SD of 4 different samples. (B) Effect of fucoidan on iNOS expression in LPS-stimulated macrophages. iNOS protein levels were monitored 18 h after treating cells with LPS (1 μg/ml). (C) Effect of fucoidan on the transactivation of *iNOS* gene. Induction of luciferase activity by LPS in the RAW264.7 cells transiently transfected with pGL-miNOS1588 construct, which contained −1588 bp iNOS promoter sequences, was confirmed using a luminometer. A dual luciferase reporter gene assay was performed on the lysed cells co-transfected with pGL-miNOS1588 (firefly luciferase) and pRL-SV (*Renilla* luciferase) (in a ratio of 250:1) after exposure to LPS (1 μg/ml) and fucoidan (30 and 100 μg/ml) for 18 h. The activation of the reporter gene was calculated as a relative change in the *Renilla* luciferase activity. Data represent means ± SD of 4 separate samples (significant versus the control, $**p < 0.01$; significant versus the LPS-treated group, $^{\#\#}p < 0.01$).

inhibitory subunit, I-κBα, is phosphorylated and degraded, liberating an active p65, which is then translocated into the nucleus [14]. Therefore, the nuclear p65 levels were measured to confirm the result of the NF-κB reporter gene

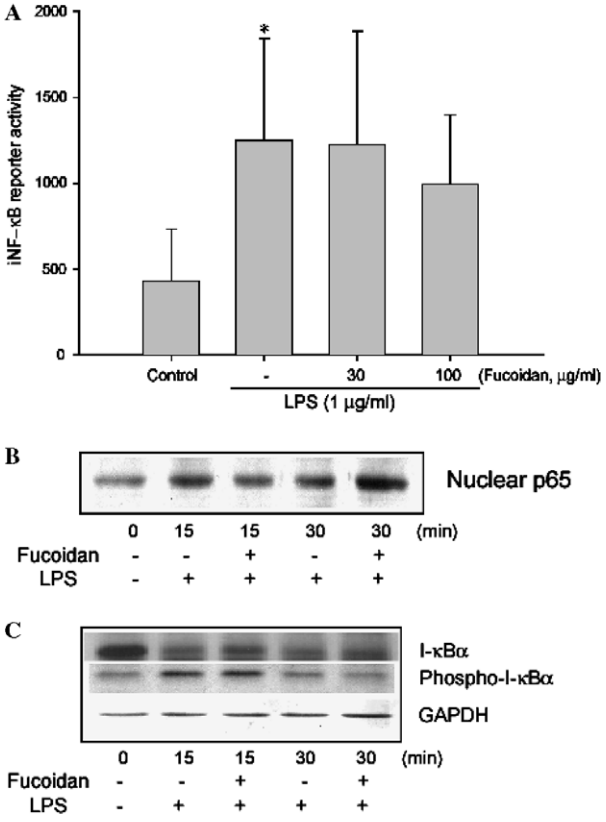

Fig. 3. Effect of fucoidan on NF-κB activation. (A) NF-κB reporter gene analysis. Cells were transfected with pNF-κB-Luc plasmid, and reporter gene analysis was performed as described in the legend of Fig. 2, panel (C). Data represent means ± SD of 5 or 7 separate samples (significant versus the control, $*p < 0.05$). (B) Effects of fucoidan on the LPS-induced nuclear translocation of p65. RAW264.7 cells were treated with 1 μg/ml LPS for 15 min or 30 min in the presence or absence of 100 μg/ml fucoidan, and nuclear p65 protein was detected immunochemically using anti-p65 antibody. Equal loadings of nuclear protein were verified by Ponceau-S staining. (C) Effect of fucoidan on LPS-induced I-κBα phosphorylation and degradation. The phosphorylation and degradation of I-κBα were immunochemically assessed by treating cells preincubated with 100 μg/ml fucoidan for 10 min with LPS.

assay. The p65 protein levels in the nuclear fractions were increased 15 or 30 min after the LPS (1 μg/ml) treatment, and were not lower in the cells pretreated with 100 μM fucoidan (Fig. 3B).

The protein levels of I-κBα and phosphorylated I-κBα in macrophages were also assessed. Immunoblot analysis using the specific antibodies revealed that 100 μM fucoidan did not reverse the phosphorylation and subsequent degradation of I-κBα by LPS (1 μg/ml) (Fig. 3C). These results show that NF-κB activation by LPS was unaffected by the fucoidan treatment.

Inhibitory effect of fucoidan on LPS-inducible AP-1 activation

The expression of the *iNOS* gene is also controlled by the transcription factor, AP-1 [15–17]. The level of AP-1 activation was assessed by a reporter gene assay using the luciferase plasmid containing the AP-1 consensus sequences. Fucoidan (30 or 100 μM) significantly inhibited the LPS-induced increases in AP-1 reporter activity (Fig. 4A). This suggests that blocking AP-1 activation may be the mechanistic basis for the inhibition of iNOS expression by fucoidan.

In order to confirm fucoidan's inhibitory effect on AP-1 activation, gel shift analysis was performed using a radiolabeled AP-1 consensus sequence. The band intensity of the slow migrating complex was increased 1 h after the LPS treatment, which confirmed that AP-1 was activated by 1 μg/ml LPS (Fig. 4B). The addition of a 20-fold excess of an unlabeled AP-1 to the nuclear extract obtained from the LPS-stimulated cells abolished the AP-1 binding (Fig. 4B). Fucoidan (30 or 100 μM) inhibited the

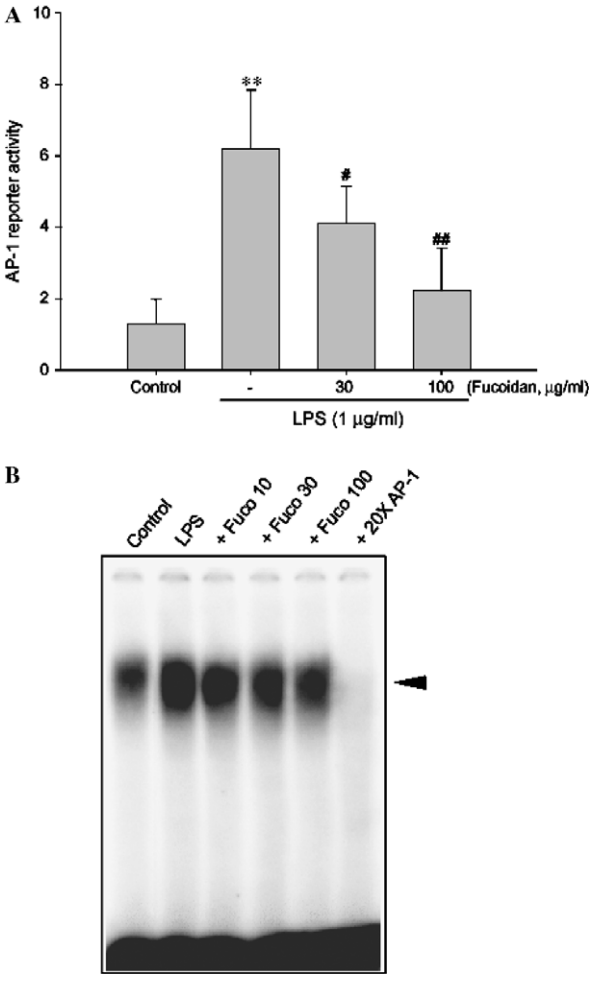

Fig. 4. Effect of fucoidan on AP-1 activation. (A) AP-1 reporter gene assay. Cells were transfected with pAP-1-Luc plasmid, and reporter gene analysis was performed as described in the legend of Fig. 2, panel (C). Data represent means ± SD of 4 separate samples (significant versus the control, $**p < 0.01$; significant versus the LPS-treated group, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$). (B) Gel shift analyses of AP-1. Gel shift assays were performed with nuclear extracts prepared from RAW264.7 cells cultured with or without LPS (1 μg/ml) and fucoidan (10–100 μg/ml) for 1 h. All lanes were loaded with 10 μg of nuclear extracts and labeled AP-1 DNA consensus sequence. The arrowhead indicates the AP-1 binding complex.

LPS-inducible AP-1 DNA binding, with complete inhibition being observed at 100 μM of fucoidan (Fig. 4B).

Discussion

The production of excess NO by iNOS induction in activated macrophages plays a key role in severe inflammatory diseases such as sepsis and arthritis [18,19]. In particular, NO induces the collapse of the vascular reactivity and causes pathological alterations [20]. Therefore, the selective inhibition of iNOS expression in inflammatory cells, such as macrophages, may offer a new therapeutic strategy against inflammation.

MSR1 or the class A scavenger receptor on macrophages or transformed foam cells in atherosclerotic lesions is involved in internalizing the modified lipoproteins, and is believed to be one of the pathological mediators during atherosclerosis progression [21,22]. Fucoidan is recognized as a ligand for MSR1, and can be taken up by macrophages via an endocytosis-dependent mechanism [6]. It was recently reported that an interaction between macrophages and fucoidan causes the production of NO via a p38 kinase and NF-κB-dependent mechanism [6]. In contrast, we found for the first time that fucoidan, a main constituent of brown algae, inhibits the expression of iNOS in LPS-activated macrophages via the selective blocking of AP-1 activation. Fucoidan has several biological activities. Recently, its anti-inflammatory and anti-complement actions have attracted considerable attention [1]. The unique suppressing effects on iNOS induction in activated macrophages might be one of explanations for its anti-inflammatory actions.

Inflammatory cytokines such as TNF-α exert their biological effects through members of the cytokine receptor superfamily, whereas, LPS triggers the initial signals via TLR4 [11]. Hence, the mechanisms for regulating the induction of iNOS by LPS appear to be different from those caused by inflammatory cytokines. We also found that fucoidan blocked iNOS expression in TNF-α-stimulated macrophages (data not shown). This suggests that fucoidan may not act on the extracellular receptors but on the intracellular signaling machinery such as the process of transcription factor activation.

The *iNOS* gene promoter contains several homologous consensus sequences for binding the transcription factors such as NF-κB, AP-1, and C/EBP [23,24], and NF-κB and AP-1 are believed to be essential for *iNOS* transcription [25,26]. In this study, reporter gene analysis using the NF-κB minimal promoter and Western blot analysis using p65, I-κBα, and phospho-I-κBα antibodies, revealed that fucoidan minimally affected the activation of NF-κB by LPS. However, the AP-1 reporter gene and gel shift analyses (Fig. 4A and B) revealed that LPS-induced AP-1 activation was suppressed by fucoidan. These results suggest that the inhibitory effect of fucoidan on the induction of iNOS in activated macrophages is closely related to the blocking of inflammatory signal(s)-inducible AP-1 activation. The most representative physiological effect of fucoidan is the anti-coagulation effect, which is due to its structure similarity to heparin [27]. Heparin can migrate into the nucleus and suppress AP-1-mediated transcription in smooth muscle cells and hepatoma cells [28,29]. Hence, fucoidan may freely translocate to the nucleus and affect the activity of some transcription factors.

The activation status of a variety of transcription factors is controlled by the generation of ROS [30], and the activation of AP-1 is dependent on the cellular redox status [31,32]. Recently, it was suggested that fucoidan acts as a potential anti-oxidant [33] and it was also found that the sulfated polysaccharide efficiently scavenges hydroxyl and peroxyl radicals as well as peroxynitrite ions in the Total Oxidant Scavenging Capacity (TOSC) assay system (Kim SK et al., unpublished data). Hence, the blocking of AP-1 activation by fucoidan in activated macrophages may be associated with its anti-oxidant effect.

In conclusion, fucoidan inhibits nitric oxide production in macrophages activated by LPS or TNF-α through the selective inhibition of AP-1 activation. This inhibition can explain some of the anti-inflammatory effects of fucoidan.

Acknowledgment

This work was supported by research fund from Chosun University (2006).

References

[1] O. Berteau, B. Mulloy, Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide, Glycobiology 13 (2003) 29R–40R.
[2] C. Blondin, F. Chaubet, A. Nardella, C. Sinquin, J. Jozefonvicz, Relationships between chemical characteristics and anticomplementary activity of fucans, Biomaterials 17 (1996) 597–603.
[3] G. Linnemann, K. Reinhart, U. Parade, A. Philipp, W. Pfister, E. Straube, W. Karzai, The effects of inhibiting leukocyte migration with fucoidin in a rat peritonitis model, Intensive Care Med. 26 (2000) 1540–1546.
[4] D. Logeart, S. Prigent-Richard, J. Jozefonvicz, D. Letourneur, Fucans, sulfated polysaccharides extracted from brown seaweeds, inhibit vascular smooth muscle cell proliferation. I. Comparison with heparin for antiproliferative activity, binding and internalization, Eur. J. Cell Biol. 74 (1997) 376–384.
[5] H.Y. Hsu, S.L. Chiu, M.H. Wen, K.Y. Chen, K.F. Hua, Ligands of macrophage scavenger receptor induce cytokine expression via differential modulation of protein kinase signaling pathways, J. Biol. Chem. 276 (2001) 28719–28730.
[6] T. Nakamura, H. Suzuki, Y. Wada, T. Kodama, T. Doi, Fucoidan induces nitric oxide production via p38 mitogen-activated protein kinase and NF-kappaB-dependent signaling pathways through macrophage scavenger receptors, Biochem. Biophys. Res. Commun. 343 (2006) 286–294.
[7] K.W. Kang, S.Y. Choi, M.K. Cho, C.H. Lee, S.G. Kim, Thrombin induces nitric-oxide synthase via Galpha12/13-coupled protein kinase C-dependent I-kappaBalpha phosphorylation and JNK-mediated I-kappaBalpha degradation, J. Biol. Chem. 278 (2003) 17368–17378.
[8] K.W. Kang, M.J. Jin, H.K. Han, IGF-I receptor gene activation enhanced the expression of monocarboxylic acid transporter 1 in

hepatocarcinoma cells, Biochem. Biophys. Res. Commun. 342 (2006) 1352–1355.
[9] E.R. Woo, J.Y. Lee, I.J. Cho, S.G. Kim, K.W. Kang, Amentoflavone inhibits the induction of nitric oxide synthase by inhibiting NF-kappaB activation in macrophages, Pharmacol. Res. 51 (2005) 539–546.
[10] U. Forstermann, H. Kleinert, Nitric oxide synthase: expression and expressional control of the three isoforms, Naunyn. Schmiedebergs Arch. Pharmacol. 352 (1995) 351–364.
[11] J.C. Chow, D.W. Young, D.T. Golenbock, W.J. Christ, F. Gusovsky, Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction, J. Biol. Chem. 274 (1999) 10689–10692.
[12] J.M. Muller, H.W. Ziegler-Heitbrock, P.A. Baeuerle, Nuclear factor kappa B, a mediator of lipopolysaccharide effects, Immunobiology 187 (1993) 233–256.
[13] M. Guha, N. Mackman, LPS induction of gene expression in human monocytes, Cell. Signal. 13 (2001) 85–94.
[14] T.D. Gilmore, The Rel/NF-kappaB signal transduction pathway: introduction, Oncogene 18 (1999) 6842–6844.
[15] M.K. Cho, S.H. Suh, S.G. Kim, JunB/AP-1 and NF-kappa B-mediated induction of nitric oxide synthase by bovine type I collagen in serum-stimulated murine macrophages, Nitric Oxide 6 (2002) 319–332.
[16] C.W. Chen, S.T. Lee, W.T. Wu, W.M. Fu, F.M. Ho, W.W. Lin, Signal transduction for inhibition of inducible nitric oxide synthase and cyclooxygenase-2 induction by capsaicin and related analogs in macrophages, Br. J. Pharmacol. 140 (2003) 1077–1087.
[17] D.Y. Choi, J.Y. Lee, M.R. Kim, E.R. Woo, Y.G. Kim, K.W. Kang, Chrysoeriol potently inhibits the induction of nitric oxide synthase by blocking AP-1 activation, J. Biomed. Sci. 12 (2005) 949–959.
[18] C. Szabo, Role of nitric oxide in endotoxic shock. An overview of recent advances, Ann. N Y Acad. Sci. 851 (1998) 422–425.
[19] J.B. Weinberg, Nitric oxide synthase 2 and cyclooxygenase 2 interactions in inflammation, Immunol. Res. 22 (2000) 319–341.
[20] S. Symeonides, R.A. Balk, Nitric oxide in the pathogenesis of sepsis, Infect. Dis. Clin. North Am. 13 (1999) 449–463.
[21] S. Parthasarathy, L.G. Fong, D. Otero, D. Steinberg, Recognition of solubilized apoproteins from delipidated, oxidized low density lipoprotein (LDL) by the acetyl-LDL receptor, Proc. Natl. Acad. Sci. USA 84 (1987) 537–540.
[22] E. Ottnad, S. Parthasarathy, G.R. Sambrano, M.P. Ramprasad, O. Quehenberger, N. Kondratenko, S. Green, D. Steinberg, A macrophage receptor for oxidized low density lipoprotein distinct from the receptor for acetyl low density lipoprotein: partial purification and role in recognition of oxidatively damaged cells, Proc. Natl. Acad. Sci. USA 92 (1995) 1391–1395.
[23] K. Cieslik, Y. Zhu, K.K. Wu, Salicylate suppresses macrophage nitric-oxide synthase-2 and cyclo-oxygenase-2 expression by inhibiting CCAAT/enhancer-binding protein-beta binding via a common signaling pathway, J. Biol. Chem. 277 (2002) 49304–49310.
[24] A.K. Lee, S.H. Sung, Y.C. Kim, S.G. Kim, Inhibition of lipopolysaccharide-inducible nitric oxide synthase, TNF-alpha and COX-2 expression by sauchinone effects on I-kappaBalpha phosphorylation, C/EBP and AP-1 activation, Br. J. Pharmacol. 139 (2003) 11–20.
[25] Q.W. Xie, R. Whisnant, C. Nathan, Promoter of the mouse gene encoding calcium-independent nitric oxide synthase confers inducibility by interferon-γ and bacterial lipopolysaccharide, J. Exp. Med. 177 (1993) 1779–1784.
[26] M.J. Diaz-Guerra, M. Velasco, P. Martin-Sanz, L. Bosca, Evidence for common mechanisms in the transcriptional control of type II nitric oxide synthase in isolated hepatocytes. Requirement of NF-kappaB activation after stimulation with bacterial cell wall products and phorbol esters, J. Biol. Chem. 271 (1996) 30114–30120.
[27] M.S. Pereira, B. Mulloy, P.A. Mourao, Structure and anticoagulant activity of sulfated fucans. Comparison between the regular, repetitive, and linear fucans from echinoderms with the more heterogeneous and branched polymers from brown algae, J. Biol. Chem. 274 (1999) 7656–7667.
[28] S.J. Busch, G.A. Martin, R.L. Barnhart, M. Mano, A.D. Cardin, R.L. Jackson, Trans-repressor activity of nuclear glycosaminoglycans on fos and jun/AP-1 oncoprotein mediated transcription, J. Cell Biol. 116 (1992) 31–42.
[29] J. Dudas, G. Ramadori, T. Knittel, K. Neubauer, D. Raddatz, K. Egedy, I. Kovalszky, Effect of heparin and liver heparan sulphate on interaction of HepG2-derived transcription factors and their *cis*-acting elements: altered potential of hepatocellular carcinoma heparan sulphate, Biochem. J. 350 (2000) 245–251.
[30] C.G. Pham, C. Bubici, F. Zazzeroni, S. Papa, J. Jones, K. Alvarez, S. Jayawardena, E. De Smaele, R. Cong, C. Beaumont, F.M. Torti, S.V. Torti, G. Franzoso, Ferritin heavy chain upregulation by NF-kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species, Cell 119 (2004) 529–542.
[31] S.J. Lin, S.K. Shyue, P.L. Liu, Y.H. Chen, H.H. Ku, J.W. Chen, K.B. Tam, Y.L. Chen, Adenovirus-mediated overexpression of catalase attenuates oxLDL-induced apoptosis in human aortic endothelial cells via AP-1 and C-Jun N-terminal kinase/extracellular signal-regulated kinase mitogen-activated protein kinase pathways, J. Mol. Cell. Cardiol. 36 (2004) 129–139.
[32] C.H. Woo, J.H. Lim, J.H. Kim, Lipopolysaccharide induces matrix metalloproteinase-9 expression via a mitochondrial reactive oxygen species-p38 kinase-activator protein-1 pathway in Raw 264.7 cells, J. Immunol. 173 (2004) 6973–6980.
[33] P. Ruperez, O. Ahrazem, J.A. Leal, Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*, J. Agric. Food Chem. 50 (2002) 840–845.