The Immunologically Active Oligosaccharides Isolated from Wheatgrass Modulate Monocytes via Toll-like Receptor-2 Signaling

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Background: Wheatgrass is a supplemental food that enhances immunity and improves various diseases; however, there remains a lack of scientific evidence for this.

Results: Wheatgrass-derived α-(1,4)-linked heptaglucan maltoheptaose systematically increases immune activation.

Conclusion: Maltoheptaose is an immune stimulator that activates monocytes via Toll-like receptor-2 signaling.

Significance: This is the first work to address the immunostimulatory component of wheatgrass with well defined molecular structures and mechanisms.

Wheatgrass is one of the most widely used health foods, but its functional components and mechanisms remain unexplored. Herein, wheatgrass-derived oligosaccharides (WG-PS3) were isolated and found to induce CD69 and Th1 cytokine expression in human peripheral blood mononuclear cells. In particular, WG-PS3 directly activated the purified monocytes by inducing the expression of CD69, CD80, CD86, IL-12, and TNF-α but affected NK and T cells only in the presence of monocytes. After further purification and structural analysis, maltoheptaose was identified from WG-PS3 as an immunomodulator. Maltoheptaose activated monocytes via Toll-like receptor 2 (TLR-2) signaling, as discovered by pretreatment of blocking antibodies against Toll-like receptors (TLRs) and also determined by click chemistry. This study is the first to reveal the immunostimulatory component of wheatgrass with well defined molecular structures and mechanisms.

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**The Journal of Biological Chemistry**

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observe the phenotypic changes and describe the immune modulation properties of wheatgrass.

In the present study, the structural features and bioactive immunostimulatory mechanisms of the carbohydrate components of wheatgrass are demonstrated. The results suggest that wheatgrass-derived oligosaccharides have systematic immunostimulatory bioactivity in hPBMCs through activating monocytes directly. Maltoheptaose was further purified and identified as the immunomodulatory compound to activate monocytes via TLR-2.

EXPERIMENTAL PROCEDURES

Extraction and Isolation of Wheatgrass—Wheatgrass was purchased from a vitality shop and was cut into slices and then lyophilized and ground into powder. The 500 g of lyophilized wheatgrass powder was extracted three times with 2 liters of ethanol at 4 °C, and the resulting precipitate was named wheatgrass powder. Wheatgrass powder was extracted three times with 2 liters of water-soluble portion was precipitated by four volumes of ethanol at 4 °C, and the resulting precipitate was named wheatgrass powder. The 500 g of lyophilized wheatgrass powder was extracted three times with 2 liters of ethanol, and the concentrated extract was collected, lyophilized, and resuspended with 1 liter of distilled water at 85 °C. The water-soluble portion was precipitated by four volumes of ethanol at 4 °C, and the resulting precipitate was named wheatgrass polysaccharides (WG-PS). Wheatgrass polysaccharides (WG-PS-C) were obtained by treatment with 0.1 M TF A at 0 °C for 1 h, whereas wheatgrass polysaccharide peptides (WG-PS-P) were obtained by treatment with 0.1 M TFA at 100 °C for 1 h to remove the carbohydrates. The WG-PS sample was further chromatographed over a Bio-Gel P-6 gel filtration column (1.5 × 90 cm), which was eluted with distilled water containing 0.02% sodium azide at a flow rate of 0.5 ml/min. All chromatographic fractions containing carbohydrates were detected by phenol-sulfuric acid and quantitatively measured at an optical density (OD) of 490 nm.

Isolation and Stimulation of hPBMCs and Its Subsets—The hPBMCs were separated from buffy coats obtained from healthy donors by Ficoll-Hypaque centrifugation (GE Healthcare), in accordance with the manufacturer’s instructions. The human monocytes, NK cells, and T cells were enriched from hPBMCs by negative selection following the magnetically negative depletion protocol (Miltenyi Biotec). The purified cells were incubated for 24 h in RPMI 1640 culture conditions (37 °C and 5% CO2) to equilibrate and were then stimulated with various treatments. LPS (Escherichia coli 0111:B4) as a positive control was treated at 1 µg/ml. An equal volume of 1% SDS and 1% sodium dodecyl sulfate (SDS-PAGE) were used for the synthesis of probe-modified maltoheptaose are available (see supplemental Fig. S7 and supplemental Experimental Procedures). THP-1 cells (106 cells) were untreated and treated with maltoheptaose, maltoheptaose-probe, or probe alone at a concentration of 8.67 mM in PBS for 2 h. After the reaction, a photochemical reactor, PR-2000 (PANCHUM, Taiwan), was applied to perform diazirine photo-cross-linking in the source of a 100-watt mercury lamp for irradiation at 352 nm for 15 min.

Flow Cytometry—Flow cytometry analysis was performed according to standard procedures. The following antibodies were used: anti-CD3, anti-CD14, anti-CD19, anti-CD56, anti-CD69, anti-CD80, and anti-CD86. Fluorescence-conjugated antibodies were purchased from BioLegend. Single cell suspensions of peripheral blood were subsequently analyzed or sorted on a FACSCalibur flow cytometer (BD Biosciences) and used for cytokinofluorometric analysis. The statistical markers were set using the irrelevant isotype-matched controls as a reference.

ELISA—Levels of human IL-12, TNF-α, IL-2, and IFN-γ in a conditioned medium were collected and measured using commercially available ELISA kits (Bender Medsystems). The cytokine concentration was quantified using the color intensity from a microplate reader (Maxi II DYNEX, Hp DJ610) at an OD of 450 nm. The neutralizing antibodies specific to human TLR4/MD-2 (clone MTS510; IMGENEX), TLR-2 (clone TL2.1; IMGENEX), Dectin-1 (R&D Systems), and CR3 (BioLegend) were pretreated, and cytokine expression was analyzed.

The Separation of 2-Aminobenzamide (2-AB)-labeled Oligosaccharides—The 40-mg lyophilized glycocalyx pool derived from WG-PS3 was dissolved in 0.2 M 2-AB and 0.6 M sodium cyanoborohydride in acetic acid/DMSO (3:7, v/v) for 3 h at 65 °C. After the reaction, labeled glycans were purified by GlycoClean S cartridges (ProZyme). Next, the 2-AB-labeled WG-PS3 was profiled on a TS Kel Amide-80 normal phase HPLC column (7.8 mm × 30 cm, 10-µm particle size; TOSOH Bioscience). A 100-min gradient of 50 mM ammonium acetate, pH 4.75, in acetonitrile (20–24%) was used, followed by a 100-min gradient to 48% 50 mM ammonium acetate. The signals were detected by a Waters fluorescence detector (λex 330 nm, λem 420 nm).

NMR Spectral Analysis—The NMR spectra were recorded on a Bruker ADVANCE 600 MHz NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) with 5-mm cryoprobe DCl-4H/13C. Samples were dissolved in 1 ml of D2O. Chemical shifts are given in δ values relative to HOD signal (δH 4.8 at 25 °C) or the H-1/C-1 signals of manno pyranoside (δH 4.71/δC 100.6).

Quantitative Real-time PCR—Total RNA was extracted with TRizol reagent (Invitrogen), and then 2 µg of RNA was reverse-transcribed into cDNA using Superscript II Reverse Transcriptase (Invitrogen). The LightCycler DNA Master SYBR Green I kit (Roche Applied Science) was used for PCR amplification, and the cycling conditions were as follows: 95 °C for 10 min and then 45 cycles of 95 °C for 10 s, 60 °C for 5 s, and 72 °C for 4 s. The mRNA level of each sample was normalized to GAPDH, and the data are presented as the fold induction of mRNA expression relative to the control samples.

Diazirine Photo-cross-linking and Click Chemistry—Detailed experimental procedures for the synthesis of probe-modified maltoheptaose are available (see supplemental Fig. S7 and supplemental Experimental Procedures). THP-1 cells (105 cells) were untreated and treated with maltoheptaose, maltoheptaose-probe, or probe alone at a concentration of 8.67 mM in PBS for 2 h. After the reaction, a photochemical reactor, PR-2000 (PANCHUM, Taiwan), was applied to perform diazirine photo-cross-linking in the source of a 100-watt mercury lamp for irradiation at 352 nm for 15 min.

NF-kB Activation Assay—THP-1 cells (106 cells) in 24-well plates were transiently transfected with 1 µg of the NF-κB luciferase reporter plasmid. Cells were then treated with or without 86.7 µM maltoheptaose, 1 µg/ml LPS as a positive control. After 24 h, cells were lysed with 1× cell culture lysis buffer (Promega),
and 20 μl of cell lysates was dispensed into a 96-well plate, followed by the addition of 100 μl of luciferase assay substrate (Promega). The luciferase activity was measured as relative luminescence units, and Western blot analysis for the total cell protein amount of each sample. The firefly luciferase readings were then normalized to a lysis buffer vehicle control as well as the corresponding band intensities of the samples.

**RESULTS**

**The Immunity-modulating Properties of Wheatgrass Extracts**—The immunologically active components of wheatgrass were obtained through a series of purification procedures. The sugar and protein content of crude wheatgrass extract (WG-PS) was determined by phenol-sulfuric acid and Bradford protein quantification assays to be 91.14 and 7.65%, respectively. Single staining with anti-CD69-PE antibody showed that the WG-PS-treated hPBMCs induced an increase in CD69 expression by 1.8-fold. LPS, endotoxins of *Escherichia coli*, which act as strong stimulators for lymphocyte activation, was used as a positive control.

To address the immunological activity contributed by the protein or sugar moieties, we removed the protein (WG-PS-C) and the sugar (WG-PS-P) of WG-PS by Pronase digestion and TFA hydrolysis, respectively. WG-PS-C revealed similar CD69 induction activity as WG-PS, whereas the activity was significantly decreased when the sugar components of WG-PS were destroyed (Fig. 1). To rule out the possibility of endotoxin contamination in WG-PS, we cultured the murine macrophage cell line Raw264.7 and treated it with either LPS or WG-PS in the presence of the endotoxin inhibitor polymyxin B. Comparing the induction of nitric oxide (NO) activity by LPS and WG-PS in Raw264.7 cells, we found that WG-PS significantly activated NO production in murine macrophage. This induction was not inhibited by polymyxin B in contrast to LPS (supplemental Fig. S1). The results suggest that WG-PS carbohydrates display an immunoactivity that can induce immune cell activation without endotoxin contamination.

WG-PS was then further separated by size exclusion chromatography and yielded three fractions named WG-PS1, WG-PS2, and WG-PS3 that cover the molecular mass range of 1000–2000 daltons according to the dextran standard (Fig. 2A). In these fractions, WG-PS3 had the highest induction of CD69 expression in hPBMCs (Fig. 2B).

**WG-PS3 Systematically Induces the Activation of hPBMCs**—Cytomic screening was achieved by combining the antibodies recognizing CD69 with other specific cell surface markers (CD3, T cells; CD19, B cells; CD14, monocytes/macrophages; CD56, natural killer cells). The cell populations affected by WG-PS3 in hPBMCs were measured by flow cytometry analysis. Fig. 3A shows the increase of WG-PS3-induced CD69 expression in CD3 T cells (~2.5-fold), CD56 NK cells (~2.5-fold), and CD14 monocytes (~4-fold). To confirm the immuno-stimulatory properties of WG-PS3, we used an ELISA to analyze the concentrations of major Th1 cytokines IL-12, TNF-α, IL-2, and IFN-γ in a culture medium of WG-PS3-stimulated hPBMCs. All of these cytokines were induced by over 2-fold after WG-PS3 treatment (Fig. 3B). By cytomic screening, we observed that WG-PS3 activated various immune cells, including T cells, NK cells, and monocytes, in hPBMCs. In addition, the up-regulated secretions of Th1 cytokines in hPBMCs were also observed in the presence of WG-PS3.

**WG-PS3 Directly Induces the Activation of Monocytes in hPBMCs**—In order to clarify the target cell of WG-PS3, the major cell population of hPBMCs was isolated by immunomagnetic separation and treated with WG-PS3. After WG-PS3 stimulation, CD69 expression was increased in purified CD14 monocytes (Fig. 4A). Moreover, expression levels of CD80 and CD86, which are known to be co-stimulatory molecules for T cell activation and are used as markers of activated antigen-presenting cells, were increased (Fig. 4A). WG-PS3 also up-regulated the secretion of IL-12 and TNF-α, the two major Th1 cytokines produced by antigen-presenting cells, in the culture medium of primary monocytes and monocytic cell line THP-1, respectively (Fig. 4, B and C). Interestingly, WG-PS3 did not reveal direct regulation in purified primary T cells and NK cells (supplementary Fig. S2). Thus, we further investigated the role of monocytes in T cell and NK cell activations. We removed the CD14 monocytes from hPBMC using magnetic anti-CD14 microbeads and performed cytomic screening to observe the activation of T cells and NK cells. The activation of these cells was significantly reduced in the absence of monocytes under WG-PS3 stimulation (supplemental Fig. S3A). Co-culturing the purified NK cells and T cells with monocytes in the presence of WG-PS3 showed the activation of NK and T cells with an increase in CD69 expression (supplemental Fig. S3, B and C). These results demonstrated that WG-PS3-activated monocytes played a central role in building immunity by expression of surface markers and cytokines and then modulation of the T lymphocytes and NK cell activities.

**Structural Characterization of WG-PS3**—The chemical structure and composition of WG-PS3 were determined by a series of analyses, including GC-MS sugar analysis, MS/MS, and NMR spectra. The resulting mass and MS/MS showed that
WG-PS3 was an oligomer composed of hexose without peptidyl conjugation. The difference of each major signal was 162 (native sample) and 204 (permethylated sample), which characterized the presence of hexose repeats. The $m/z$ 1293 and $m/z$ 1089 in the mass spectra of permethylated WG-PS3 were picked for MS/MS analysis, and the results confirmed that
these molecules were oligomers composed of hexose (m/z 1293 for six hexoses and m/z 1089 for five hexoses) (supplemental Fig. S4A). The sugar linkage of WG-PS3 was observed to be primarily in the 1,4-glucosidic form according to the GC-MS-PMAA method (data not shown). The anomeric configurations of WG-PS3 were assigned to include both α-form (1JH = 4 Hz at δ 5.19) and β-form (1JH = 8 Hz at δ 4.6) by the 1H NMR spectra (supplemental Fig. S4B). These results indicated that WG-PS3 was a wheatgrass-derived oligosaccharide with 1,4-glycosidic linkage.

**Maltoheptaose Is Identified from WG-PS3 with Immune Activation Properties**—In order to obtain pure molecules for structural and functional study, WG-PS3 were labeled with fluorescent 2-AB by reductive amination and further purified by HPLC. Eleven peaks were separated and collected (supplemental Fig. S5A). The functional activity of these molecules was oligomers composed of hexose (m/z 1293 for six hexoses and m/z 1089 for five hexoses) (supplemental Fig. S4A). The sugar linkage of WG-PS3 was observed to be primarily in the 1,4-glucosidic form according to the GC-MS-PMAA method (data not shown). The anomeric configurations of WG-PS3 were assigned to include both α-form (1JH = 4 Hz at δ 5.19) and β-form (1JH = 8 Hz at δ 4.6) by the 1H NMR spectra (supplemental Fig. S4B). These results indicated that WG-PS3 was a wheatgrass-derived oligosaccharide with 1,4-glycosidic linkage.

**Maltoheptaose Stimulates the Cytokine Expression in Monocytes**—According to the immunomodulating properties of WG-PS3-9 described above, we examined the stimulation activities of wheatgrass-derived maltoheptaose (maltoheptaose-2-AB) in THP-1 cells. Maltoheptaose-2-AB displayed significant IL-12 and TNF-α cytokine induction properties in THP-1 cells (data not shown). To exclude the bioactive effect of 2-AB and confirm the activity of maltoheptaose, we used commercially acquired maltoheptaose to evaluate the cytokine induction activity by ELISA and a quantitative real-time PCR assay in THP-1 cells (Fig. 6, A–D). The results showed that the production of IL-12 and TNF-α was induced by maltoheptaose in a dose-dependent manner at both protein and gene levels.

**TLR-2 Is the Target Receptor of Maltoheptaose in Monocytes**—To identify the target receptor of maltoheptaose, we pretreated the blocking monoclonal antibodies to specifically recognize the candidate receptors in cultured THP-1 cells. We then analyzed the cytokine production under maltoheptaose stimulation. The candidate receptors tested included β-glucan receptors (Dectin-1 and CR3) and Toll-like receptors (TLR-2 and TLR-4), which are implicated in the recognition of various bacterial cell wall components and are naturally derived heteroglycans and proteoglycans. Only pretreatment of the TLR-2-blocking antibody significantly affected the maltoheptaose-stimulated cytokine expression (Fig. 7A). The cytokine-inhibitory behavior of the TLR-2-blocking antibody also varied based on dosage (Fig. 7B).

To further investigate the binding of maltoheptaose with TLR2 in a cell model, THP-1 cells were treated with the probe-modified maltoheptaose, which contained diazirine and an alkylne-containing photoaffinity structure for photo-cross-linking and biotin labeling. The immunoblotting showed the streptavidin signal after treatment with the maltoheptaose probe but not with maltoheptaose or the probe alone, and the TLR2-specific antibody detected the signal at the same site consistently (Fig. 7C). TLR4-specific antibody served as a negative control to exclude the possibility of nonspecific action of the cross-linking agent (supplemental Fig. S8). In addition, the target protein was cut from the acrylamide gel and subjected to in-gel trypsin digestion for analysis by electrospray ionization quadrupole time-of-flight mass spectrometry. The resulting peptide fragments were in accordance with the TLR2 amino acid sequence (data not shown). Given that binding between maltoheptaose and TLR2 occurs to induce IL-12 and TNF-α production and it is known that NF-κB plays a crucial role in TLR2-induced cytokine expression, we verified whether malto-
FIGURE 5. The proposed structure of a-(1,4-glc)$_n$-2AB.

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Maltoheptaose induces IL-12 and TNF-α protein secretion and gene expression in THP-1 cells. A and B, THP-1 cells were treated with maltoheptaose (100 and 200 μM) for 24 h. The conditioned medium was harvested, and the concentrations of IL-12 and TNF-α were assayed by ELISA (100 μl of conditioned medium/assay). C and D, quantitative real-time PCR analysis of IL-12 and TNF-α expression in THP-1 cells after treating with maltoheptaose (100 and 200 μM) for 2, 4, 8, and 12 h. The relative quantification was determined by comparing with the control sample. The experiments were repeated three times. The results are expressed as mean ± S.E. (error bars).

FIGURE 6. Maltoheptaose induces IL-12 and TNF-α protein secretion and gene expression in THP-1 cells.
heptaose drives TLR-2 downstream NF-κB activation. THP-1 cells were transfected with NF-κB luciferase reporter and then treated with maltoheptaose and the positive control, LPS, for 24 h. As shown in Fig. 7D, the NF-κB-driven luciferase activity was much higher in cells treated with maltoheptaose and LPS than untreated control. These data suggest that maltoheptaose drives TLR-2 downstream NF-κB activation. THP-1 cells were transfected with NF-κB luciferase reporter and then treated with maltoheptaose and the positive control, LPS, for 24 h. As shown in Fig. 7D, the NF-κB-driven luciferase activity was much higher in cells treated with maltoheptaose and LPS than untreated control. These data suggest that maltoheptaose
Selective targeting of TLR-2 to activate cytokine expression in monocytes. Our findings together describe the identification and characterization of maltoheptaose isolated from wheatgrass as an immunostimulatory component that induces TLR-2 signaling in monocytes to systematically increase immune activation.

**DISCUSSION**

We report that the wheatgrass-derived oligosaccharide fraction WG-PS3 possesses systemic immunity-activating properties through directly affecting the surface antigen expression and Th1 cytokine secretion in monocytes and then indirectly activating NK and T cells only in the presence of monocytes. More importantly, maltoheptaose was identified as an immunomodulatory compound of wheatgrass to activate monocytes via TLR-2 signaling.

Immunomodulation involves complex interactions between immune cells. In order to communicate with each other and regulate the immune function, immune cells transmit the patterns of cytokine secretion and the expression of functional molecules in response to a variety of situations (14, 16). Here, the cross-talk among different cell populations in WG-PS3-treated hPBMCs was described clearly, and monocyte was the major target of WG-PS3. Maltoheptaose identified as a bioactive compound of WG-PS3 was also revealed to significantly regulate the immune properties of monocytes by inducing cytokine expression. The CD14$^+$ monocyte plays a central role in host defense and occupies more than 10% of hPBMCs (17, 18). They migrate from the bloodstream to other tissues and then differentiate into tissue-resident macrophages or dendritic cells and serve three important functions in the immune system, including phagocytosis, antigen presentation, and cytokine production. Two major cytokines were examined in this study to address functional monocytes. The first one, IL-12, is naturally produced by monocytes, dendritic cells, and macrophages when put under antigenic stimulation. It plays an important role in the activation of NK cells and T lymphocytes as well as mediating the cytotoxic activity of NK cells and CD8$^+$ cytotoxic T lymphocytes (19, 20). The other one, TNF-$\alpha$, is produced by several types of cells, especially by monocytes and macrophages. TNF-$\alpha$ mediates immune functions by inducing neutrophil proliferation during inflammation and stimulating NK cell activity. In addition, TNF-$\alpha$ causes necrosis in some types of tumor cells (21, 22). We observed that WG-PS3-activated monocytes can produce these cytokines successfully.

According to known information about cytokines, the up-regulated IL-12 in maltoheptaose-stimulated monocytes may play a major role in immunostimulation. Moreover, IL-12 has been recognized as a regulator of Th1-driven immune responses (22), including the lytic functions of cytotoxic T lymphocytes and NK cells. IL-12 also greatly enhances the capacity of T cells and NK cells to produce IL-2 and IFN-$\gamma$. This partly explains how maltoheptaose-activated monocytes affect NK and T cells and implies that maltoheptaose may have broader influences on the immune response.

The structural characteristics of the bioactive glycans identified in this study were distinguishable from the $\beta$-glucan oligosaccharide described as a potent immune stimulator (23, 24). $\beta$-Glucan is composed of $\alpha$-glucose monomers linked by glycosidic bonds with $\beta$-form anomeric configurations. It is usually found in plants, the bran of cereal grains, the cell wall of baker’s yeast, certain fungi, mushrooms, and bacteria. Most of the $\beta$-glucan derived from cereal grains, such as barley, usually occurs as $\beta$(1,3)- and $\beta$(1,4)-linked polymers. However, in fungi and yeast, $\beta$-glucan presents as $\beta$(1,3)-linked glucose polymers with $\beta$(1,6)-linked side chains of various lengths and distributions. There is evidence that (1,3/1,6)-$\beta$-glucan has greater biological activity than (1,3/1,4)-$\beta$-glucan and that the immunoregulatory ability is influenced by the polymer length, the degree of branching, and its configuration (25, 26). As for the cellular response, Dectin-1 was the receptor specific for (1,3)-$\beta$-glucan in monocytes and macrophages, and the minimum unit of recognition was thought to be a 9- or 10-mer oligosaccharide (27). Until now, studies exploring malto-oligosaccharides for their immunomodulatory properties have been rare. Previous studies have demonstrated that small malto-oligosaccharides with a degree of polymerization greater than 6 are effective at modulating the immune system through observation of the reactive oxygen species production in monocytes and neutrophils (28). The authors suggested that maltoheptaoses (degree of polymerization = 7) have a tendency to form helical structures together with the two closest ends of a glucan chain. This conformation may cause the two glucose residues to interact with the receptor effectively at the same time. In this study, we first found that $\alpha$-(1,4)-linked malto-oligosaccharides “maltoheptaose” bound to TLR-2 in monocytes and significantly modulated immune response by stimulating cytokine expression. According to the computational docking of small molecule ligands onto protein receptors, the maltoheptaose could interact with TLR-2 with high affinity through a hydrogen bond between glucose residues of the two cyclic ends from maltoheptaose and the active site of the TLR2 protein (supplemental Fig. S6B).

TLRs are highly conserved from *Drosophila* to humans and share structural and functional similarities. They respond to the presence of pathogen-associated molecules, especially the sugar components, or glycoconjugates, of a diverse group of microbes (29–31). These molecules mediate cytokine production necessary for the development of effective immunity. TLR-2 causes an increase in transcription factor NF-$\kappa$B and the production of cytokines, including IL-12 and TNF-$\alpha$, to enhance immunity in host defense and cytotoxicity. Therefore, TLR-2 was considered to be a good target of immune therapy in malignant diseases to elicit innate immunity and facilitate Th1-type immune responses (32, 33). In our study, maltoheptaose was demonstrated to be a potent ligand of TLR-2. Furthermore, we suggested that maltoheptaose strongly interacted with TLR-2 at Val-269, Met-270, Glu-281, Phe-322, Tyr-323, Phe-325, and Tyr-326 with a 207.44 kcal/mol binding energy using the Discovery studio version 2.1 prediction tool protocol and defined docking strategy (supplemental Fig. S6). These results may lead to future development of wheatgrass-derived oligosaccharides for use as agents to promote immunity. The binding site analysis could be further verified and used for chemical modifications to improve potency, selectivity, or pharmacokinetic parameters.
Acknowledgments—We thank M.-D. Tsai (Institute of Biological Chemistry, Academia Sinica) and L.-P. Ting (National Yang-Ming University) for the pNF-κB-Luc plasmid.

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