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Binding Capacity of a Barley β-D-Glucan to the β-Glucan Recognition Molecule Dectin-1

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To clarify whether barley β-glucans exhibit their biological effects via binding to dectin-1, a pivotal receptor for β-1,3-glucan, the structure of barley β-glucan E70-S (BBG-70) was unambiguously investigated by NMR spectroscopy and studied for its binding capacity and specificity to dectin-1 by ELISA. NMR spectroscopy confirmed that BBG-70 contains two different linkage glucans, namely, α-glucan and β-glucan, which are not covalently attached to one another. β-Glucan within BBG-70 is a linear mixed-linkage β-glucan composed of 1,3- and 1,4-β-D-glucopyranose residues but does not contain the continuous 1,3-linkage. Competitive ELISA revealed that highly purified barley β-glucan E70-S (pBBG-70) inhibits the binding of soluble dectin-1 to sonifilan (SPG), a β-1,3-glucan, although at a concentration higher than that of SPG and laminarin. It was found that barley β-glucan can be recognized by dectin-1, implying that barley β-glucan might, at least in part, exhibit its biological effects via the recognition by dectin-1 of the ligand sugar structure, which may be formed by 1,3-β- and 1,4-β-glucosyl linkage.

KEYWORDS: NMR; barley β-glucan; dectin-1; structure

INTRODUCTION

β-Glucan is a well-known biological response modifier (BRM) widely distributed in nature. A variety of β-glucans have been isolated from various sources, for example, fungi, plants, and seaweeds. The physicochemical properties of β-glucans differ according to their primary structure, including linkage type; degree of branching (DB); degree of polymerization (DP); conformation, for example, triple helix, single helix, and random coil structures; and molecular weight (*1–3*). Recent papers have highlighted a significant role of β-glucans (1) in the treatment of cancer and infectious diseases in both modern medicine and traditional oriental therapies and (2) as a dietary substance because it lowers the plasma cholesterol level, enhances the hematopoietic response, and possesses antitumor and immunomodulating properties (*4–6*).

Host molecules that serve as β-glucan receptors have recently been reported, for example, complement receptor type 3 (CR3) (*7*) and lactosylceramide (LacCer) (*8*). More recently, dectin-1 has also been described as a β-1,3-glucan receptor (*9*). Dectin-1 is preferentially expressed on macrophages rather than granulocytes (*10*). Moreover, dectin-1 is a pivotal receptor for β-1,3-glucan because (1) it is required for the production of cytokines and reactive oxygen species (ROS) from both dendritic cells (DCs) and macrophages after stimulation with β-1,3-glucans; (2) it is required for DC maturation that is induced by a soluble β-glucan, namely, SCG, which is derived from a mushroom; and (3) it is required for optimal host defense against fungi (*11*). Therefore, dectin-1 is considered to play a major role in the immunostimulatory effects of β-1,3-glucans. It is important to know the relationship between the structure and receptor-binding ability of β-glucans (*12*).

We have also previously reported that the anti-β-glucan antibody shows reactivity to the cell wall β-glucan and whole cells of the pathogenic fungus *Candida*. It also enhances the candidacidal activity of macrophages in vitro (*13*). Thus, the antibodies against fungal β-glucan also play pivotal roles in the immunoresponse against pathogenic fungi. Very recently, we established monoclonal antibodies (Mabs) against yeast β-glucan and investigated their binding specificities to various types of β-glucans. These Mabs are expected to serve as biological probes for obtaining structural information of various β-glucans.

In addition to fungal, seaweed-derived, and bacterial β-glucans, cereal barley β-glucans, which are considered to be mixed-linkage β-glucans comprising 1,3- and 1,4-β-D-glucopyranose polymers (*14–20*), also exhibit immunostimulatory effects (*21*). The mechanisms of action as well as the structure of fungal β-glucans, which are mainly composed of 1,3- and 1,6-β-D-glucans, have been investigated extensively. However, only a

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Table 1. Primary Structures of β-Glucans Analyzed in This Study

glucan	source	primary structure	ref
SPG	*Schizophyllum commune*	1,6-β-monoglucopyranosyl branched 1,3-β-D-glucan	*28*
CSBG	*Candida albicans*	1,6-β-long glucopyranosyl branched 1,3-β-D-glucan	*22*
laminarin	*Laminaria digitata*	linear 1,3-β- and 1,6-β-glucan	*29*
dextran	*Leuconostoc dextranium*	1,3-α-glucopyranosyl branched 1,6-α-glucan	*30*

few investigations have been conducted on the mechanisms of action and structure of cereal barley β-glucans. With regard to the mechanisms of action, signal transduction through receptor–ligand interactions, in particular, remains unclear. In addition, structural analyses of cereal barley β-glucans have been conducted previously by 1D ^{13}C NMR spectroscopy on the enzyme-digested oligosaccharide derivatives (*18, 20*) and by methylation analysis (*19*). 1D ^{13}C NMR spectra are often used for the structural analysis of β-glucans because of the better signal dispersion compared with 1D ^{1}H NMR spectroscopy. However, the acquirable structural information is limited because of its inherently low sensitivity and loss of coupling information. Thus, an additional analysis using 1D ^{1}H NMR and both homo- and heteronuclear 2D NMR is vital for accurate structural elucidation of cereal barley β-glucan in order to understand its mechanisms of action. It is well established that the biological effects of β-glucan depend on its primary structure, conformation, and molecular weight (*1, 2*). Therefore, characterization of the structure of cereal barley β-glucan and its interaction with host molecules is important not only to promote its clinical usage for immunotherapy but also to understand the mechanisms underlying its biological effects.

In the present study, we report the binding capacity of barley β-glucan, which was well characterized by NMR, to the representative mammalian 1,3-β-glucan receptor, namely, dectin-1, and Mabs against yeast β-glucan.

MATERIALS AND METHODS

Materials. Barley β-glucan E70-S (BBG-70), which is currently available commercially as a health food supplement, was provided by ADEKA Co. (Tokyo, Japan). Sonifilan (schizophyllan glucan, SPG) was purchased from Kaken Pharmaceutical Co. Ltd. (Tokyo, Japan). Barley β-glucan (BBG-S) and laminarin were purchased from Sigma. Dextran T500 was purchased from GE Healthcare. β-Glucan from *Candida albicans* (CSBG) was prepared as described previously (*22*). The structures of β-glucans analyzed in this study are shown in **Table 1**. D_2O (minimum deuteration degree, of 99.96%) and Me_2SO-d_6 (99.96%) were purchased from Merck.

Purification of Barley β-Glucan. Barley β-glucan was purified according to the method of Keith et al. (U.S. Patents 147991 and 312969). Briefly, 10 g of BBG-70 was dissolved in 100 mL of distilled water in a boiling water bath. After centrifugation for 10 min at 10000*g* at 20 °C, the supernatant was collected. This supernatant was then frozen overnight at −20 °C. After melting at room temperature, the sediment was collected by centrifugation. These procedures were repeated once. The obtained sediment was then lyophilized, and purified BBG-70 (pBBG-70) was obtained (yield, 57%; purity, 91.9%). The purity of β-glucan was confirmed using the (1,3), (1,4) β-D-glucan assay kit (Megazyme, Wicklow, Ireland).

NMR Spectroscopy. The exchangeable protons were removed by preparing a suspension of barley β-glucan in D_2O and lyophilization. This exchange process was repeated three times. All spectra were recorded in 15 mg/mL of a mixed solvent, that is, Me_2SO-d_6/D_2O (6:1), at 70 °C by using a Bruker Avance 500 spectrometer equipped with a TXI-*xyz* three-gradient probe for detection of ^{1}H or a BBO-*z* gradient probe for ^{13}C. Chemical shifts are expressed as parts per million using the internal Me_2SO signal ($\delta_H = 2.53$) and internal Me_2SO ($\delta_C = 39.5$) as references for ^{1}H and ^{13}C, respectively. The 1D ^{1}H experiment was performed using the Bruker standard pulse sequence. To accurately calculate signal integrations, a relaxation delay of 5T1 was used. The 1D ^{13}C, 2D ^{1}H,^{1}H correlation spectroscopy (COSY), 2D nuclear Overhauser effect spectroscopy (NOESY), 2D ^{13}C-edited heteronuclear single quantum coherence spectroscopy (HSQC), 2D ^{1}H,^{13}C heteronuclear multiple bond coherence spectroscopy (HMBC), and 2D ^{1}H,^{13}C HSQC-total correlation spectroscopy (TOCSY) experiments were performed using the Bruker standard pulse sequence. 2D TOCSY was carried out with a spinlock mixing time of 30−150 ms using the pulse sequence of Griesinger et al. to suppress the ROE signals (*23*). 2D NOESY was conducted with a mixing time of 200 ms. 2D ^{1}H,^{13}C HMBC was performed using the delay time for evolution of long-range coupling set at 62.5 ms (optimized for 8 Hz). 2D ^{1}H,^{13}C HSQC-TOCSY was carried out with a spinlock mixing time of 30−120 ms. 2D heteronuclear two-bond correlation spectroscopy (H2BC) was carried out using the pulse sequence of Nyberg et al. (*24–26*). 2D diffusion-ordered spectroscopy (DOSY) was carried out by recording 8 scans for each gradient step with the ledbpg2s pulse sequence, a

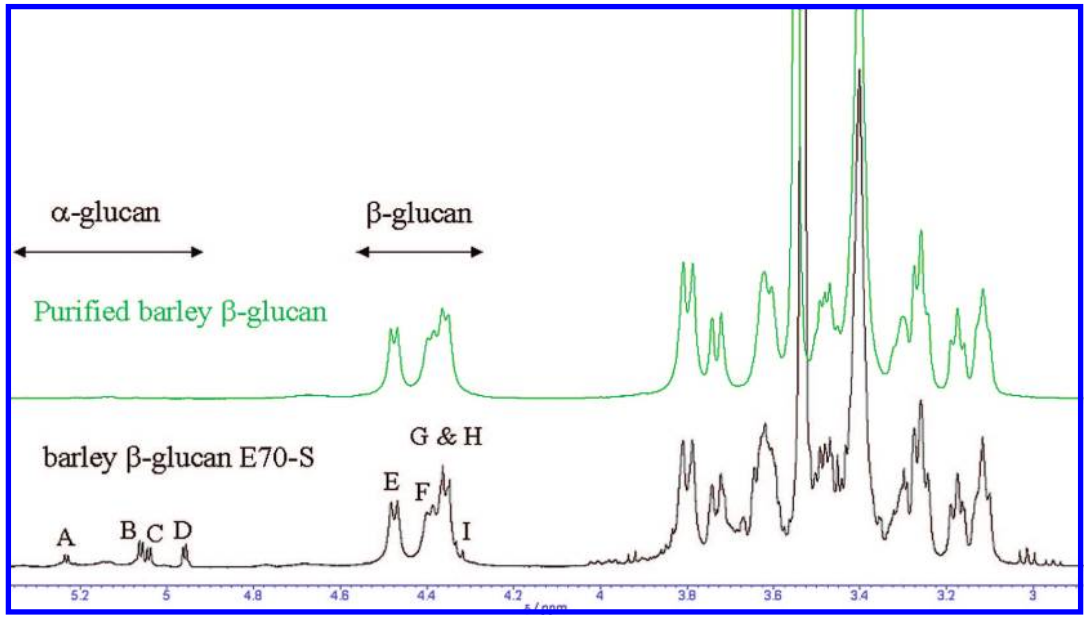

Figure 1. 1D ^{1}H NMR spectra of barley β-glucan E70-S (bottom) and purified barley β-glucan E70-S (top) at 70 °C.

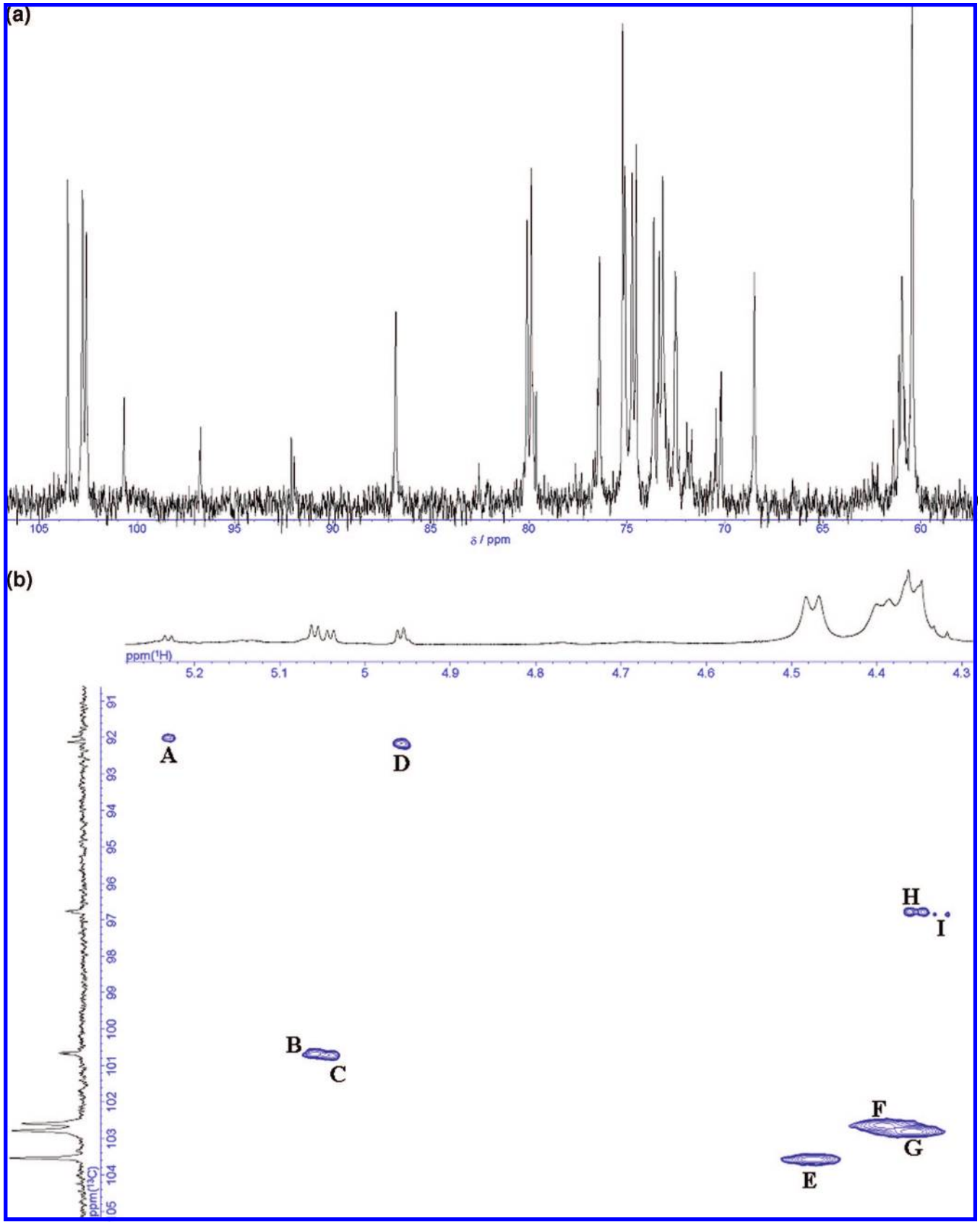

Figure 2. 1D ^{13}C NMR (**a**) and anomeric region of ^{13}C-edited HSQC spectra of barley β-glucan E70-S (**b**) at 70 °C.

linear gradient of 64 steps between 2 and 95%, a diffusion time of 100 ms, and a 3 ms length of the square diffusion encoding gradient pulses. The standard Bruker protocol was used for processing in XwinNMR software. All 2D experiments were zero-filled to 2K and 2K in both dimensions prior to Fourier transformation. A squared cosine-bell window function was applied in both dimensions in all experiments, except in the COSY and H2BC experiments. A sine-bell window function was applied in both dimensions for the COSY experiment. The H2BC experiment was treated with a cosine window function in t_1 and a $\pi/4$ shifted sine in t_2.

Table 2. Coupling Constants ($^1J_{H1,C1}$ and $^3J_{H1,H2}$) for Barley β-Glucan E70-S

residue	δ_H	$^1J_{H1,C1}$ (Hz)	$^3J_{H1,H2}$ (Hz)
A	5.231	168	3.9
B	5.059	168	3.9
C	5.041	167	3.7
D	4.958	166	3.5
E	4.476	161	7.5
F	4.393	160	7.2
G	4.359	161	7.4
H	4.355	157	7.7
I	4.325	158	7.7

Preparation of Soluble Dectin-1 Molecules. We prepared two different forms of recombinant dectin-1 molecules, namely, soluble dectin-1 (sDectin-1) and soluble dectin-1-Fc (sDectin-1-Fc). Soluble Dectin-1 was isolated from a culture supernatant of CHO cells transduced with mouse dectin-1 cDNA encoding the carbohydrate recognition domain (CRD) and polyhistidine tag sequence. The glycosylated portion of sDectin-1 was subjected to periodate oxidation and cyanoborotritide reduction for biotin hydrazide conjugation. Biotin-conjugated sDectin-1 was desalted by dialysis against phosphate-buffered saline (PBS). sDectin-1-Fc was prepared as a chimeric protein of the mDectin-1 CRD and the Fc portion of human IgG1. The recombinant Fc-chimeric protein was isolated on a Hi-trap Protein A column (GE Healthcare and Biotechnology) from the culture supernatant of the 293T transfectant cells.

sDectin-1 Binding Assay. The binding specificity of various β-glucan preparations was assessed by competitive enzyme-linked immunosorbent assay (ELISA). Briefly, an ELISA plate (Nunc) was coated with 1,6-β-monoglucopyranosyl branched 1,3-β-D-glucan from *Schizophyllum commune* (SPG, 20 μg/mL) dissolved in bicarbonate buffer (pH 9.6) by overnight incubation at 4 °C. The unbound SPG was washed with PBS containing 0.05% Tween 20 (PBST), and the plate was blocked with PBS containing 0.5% BSA (BPBS) by incubation for 2 h at room temperature. The various glucan samples were diluted with BPBS to achieve concentrations of 0−500 μg/mL and mixed with biotin-labeled sDectin-1 (100 ng/mL) for 1 h before addition to the SPG-coated ELISA plate. The plate containing sDectin-1 was incubated for 1 h at room temperature, washed with PBST, and further incubated with peroxidase-conjugated streptavidin (Pharmingen). The binding of sDectin-1 to solid-phase SPG was monitored using the peroxidase substrate TMB (KPL Inc.), and color development was stopped with 1 M phosphoric acid; the optical density was measured at 450 nm. To exclude the possibility that these glucan preparations nonspecifically inhibit the binding of sDectin-1 molecules to solid-phase SPG, the ELISA plate was coated with each of the various glucans, and the binding ability of sDectin-1 to the glucan preparations was tested. The ELISA plate was coated with various concentrations of glucan samples dissolved in a bicarbonate buffer by overnight incubation at 4 °C; the unbound glucans were removed by washing, and the plate was blocked with BPBS. The glucans on the ELISA plate were incubated with sDectin-1-Fc, washed, and probed with peroxidase-conjugated antihuman IgG. The binding of sDectin-1 to the glucans was monitored by a colorimetric assay using the peroxidase substrate TMB. The absorbance at 450 nm was measured using a microplate reader, MTP450 (Corona Electric).

Preparation of Anti-β-glucan Mab. Hybridoma secreting the anti-β-glucan antibody was generated after fusion of spleen cells of CSBG-immunized DBA/2 mice and myeloma cells of the murine line X63. ELISA assays were performed using various β-glucans as solid-phase antigens to select Mabs and assess their specificity.

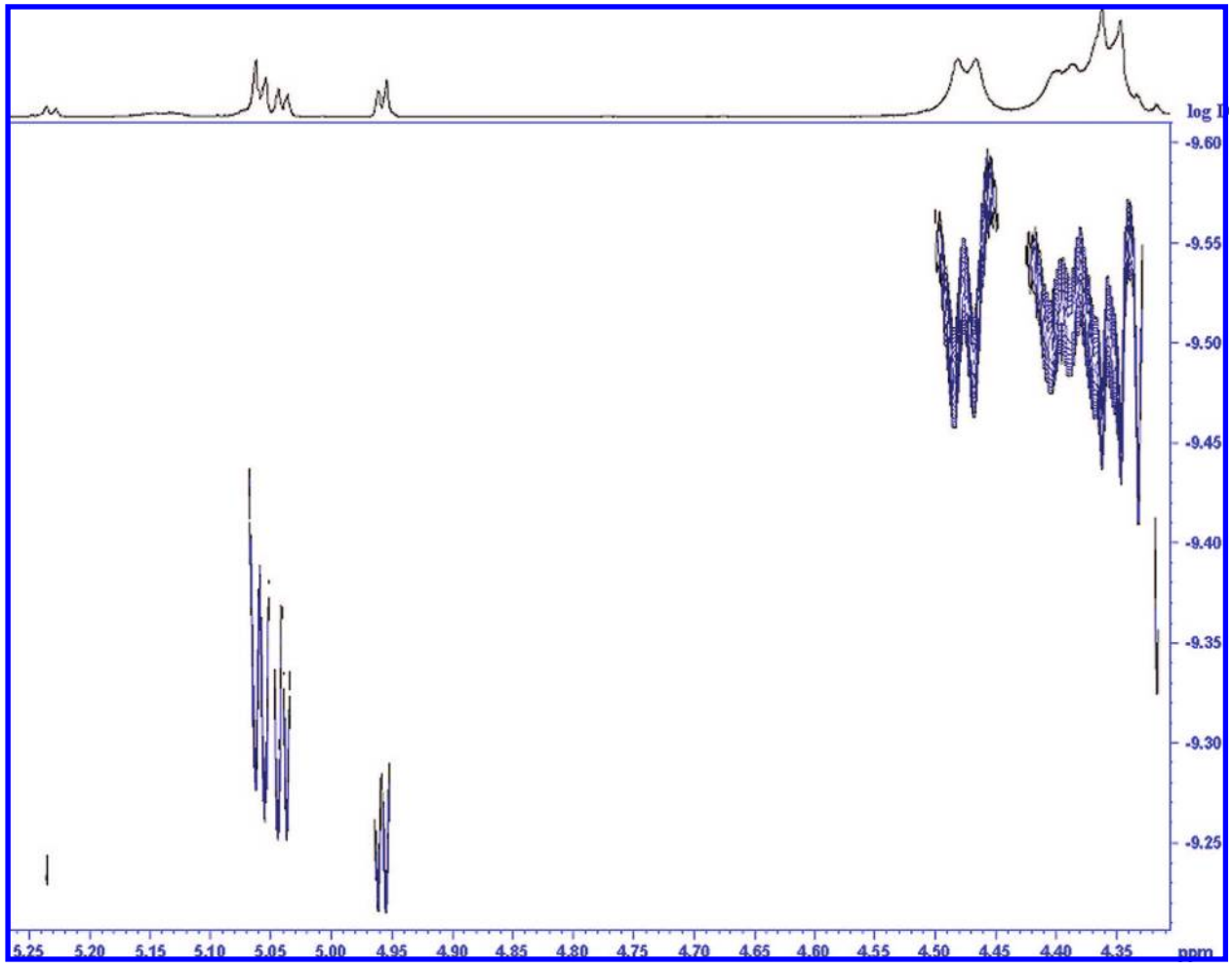

Figure 3. 2D-DOSY spectrum of barley β-glucan E70-S at 70 °C.

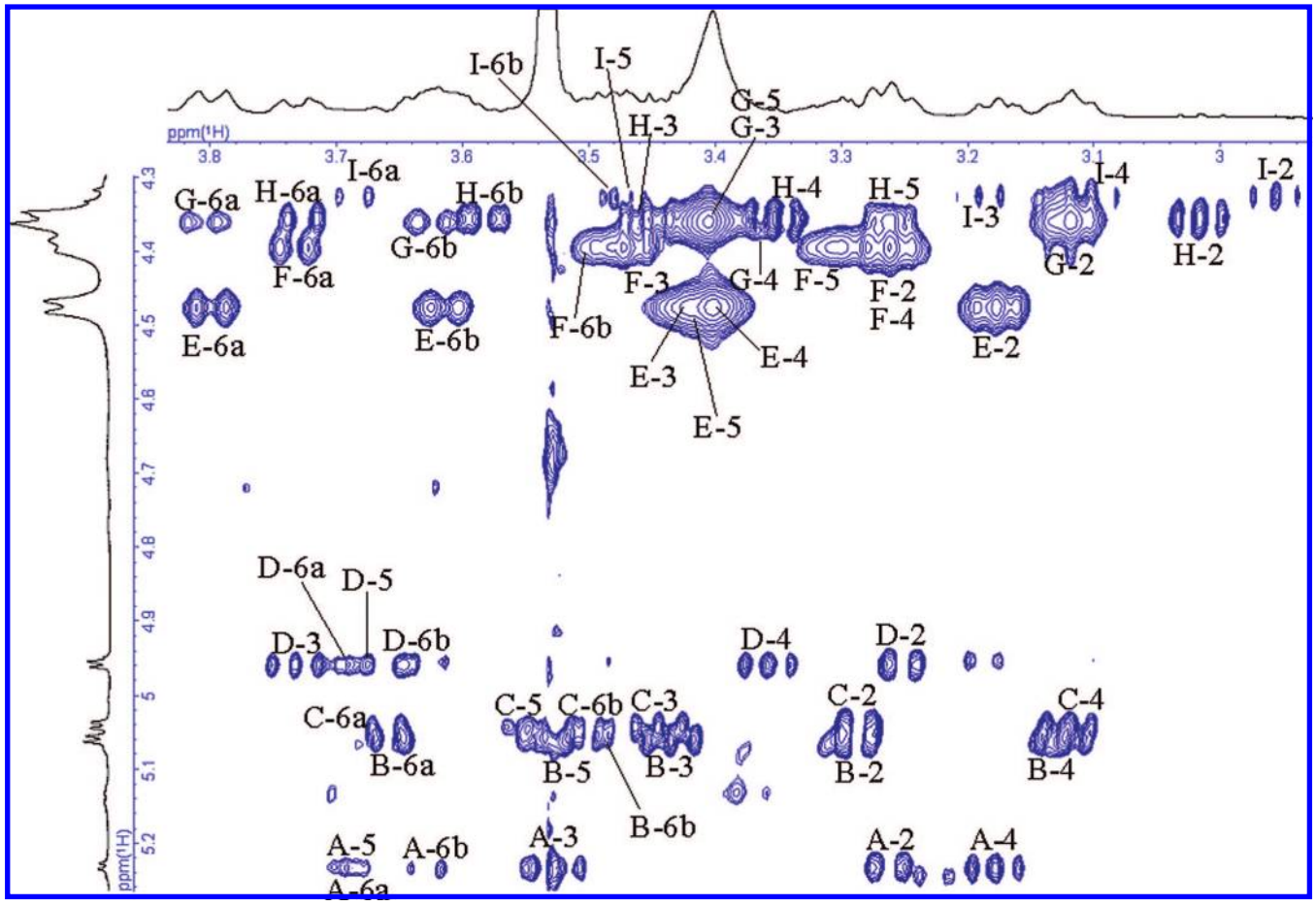

Figure 4. Anomeric region of the 2D-TOCSY spectrum of barley β-glucan E70-S at 70 °C. The 2D-TOCSY experiment was performed with a spinlock mixing time of 150 ms.

Table 3. Chemical Shifts of ^{1}H and ^{13}C for Barley β-Glucan E70-S

sugar residue	$^{1}H/^{13}C$ (ppm)							
	1	2	3	4	5	6a		6b
A	5.231	3.262	3.526	3.180	3.693	3.693		3.629
	92.01	71.74	73.09	70.19	72.91		60.84	
B	5.059	3.287	3.434	3.176	3.525	3.657		3.489
	100.67	72.55	73.42	70.19	73.39		61.41	
C	5.041	3.285	3.444	3.120	3.555	3.659		3.486
	100.71	72.58	73.44	70.22	73.41		61.23	
D	4.958	3.251	3.732	3.358	3.683	3.698		3.646
	92.17	71.95	73.04	80.11	70.48		60.84	
E	4.476	3.176	3.420	3.400	3.401	3.799		3.614
	103.58	73.66	74.72	79.90	75.19		60.46	
F	4.393	3.260	3.470	3.259	3.310	3.733		3.494
	102.66	72.56	86.82	68.53	76.42		61.09	
G	4.359	3.111	3.406	3.356	3.975	3.806		3.624
	102.80	73.22	74.72	80.11	77.31		60.93	
H	4.355	3.015	3.453	3.352	3.268	3.727		3.583
	96.79	74.54	76.51	79.69	75.25		60.99	
I	4.325	2.955	3.190	3.099	3.467	3.686		3.485
	96.86	74.94	76.65	70.58	73.21		61.44	

Mab Binding Assay Using ELISA. A 96-well Nunc plate was coated with the glucan preparation (25 μg/mL) in 0.1 M carbonate buffer (pH 9.6) and incubated overnight at 4 °C. The plate was washed with PBST and blocked with 1% BPBST at 37 °C for 1 h. After washing, the plate was incubated at 37 °C for 1 h with mouse anti-β-glucan Mab (1A5). The plate was then washed with PBST, treated with antimouse IgG+M antibody (Sigma) in BPBST, and developed using the TMB substrate system. Color development was stopped with 1 M phosphoric acid, and the optical density was measured at 450 nm.

RESULTS AND DISCUSSION

Structural Characterization by NMR Spectroscopy. To characterize the structure of a barley β-glucan used in this study (BBG-70), we first analyzed the sugar composition of alditol acetate derivative by gas–liquid chromatography (GLC); this analysis revealed that BBG-70 is mainly composed of glucose (data not shown). Next, we studied the structure of BBG-70 by NMR spectroscopy. A 1D ^{1}H NMR spectrum of a solution of Me_2SO-d_6/D_2O (6:1) at 70 °C is shown in **Figure 1** (bottom). The anomeric region (δ_H 4.3–5.3) contained nine signals, two of which heavily overlapped (δ_H 4.36). The other peak was of a doublet resonance (δ_H 4.325, 4.393, 4.476, 4.958, 5.041, 5.059, and 5.231). The overlap of the anomeric doublets was confirmed by additional NMR experiments, including a COSY experiment. The nine sugar residues in BBG-70 were arbitrarily labeled A−I, as described in **Figure 1** (bottom). The 1D ^{13}C and ^{13}C-edited HSQC spectra in **Figure 2** showed nine signals of ^{13}C in the anomeric region (δ_C 92–105) that were assigned to residues A, B, C, D, E, F, G, H, and I (δ_C 92.01, 100.67, 100.71, 92.17, 103.58, 102.66, 102.80, 96.79, and 96.86, respectively). On the basis of their observed chemical shifts, $^{3}J_{H1,H2}$ and $^{1}J_{H1,C1}$ (**Table 2**), residues A−D and residues E−I were assigned as α-anomer and β-anomer, respectively, in a ratio of 1.0:5.2 (as calculated

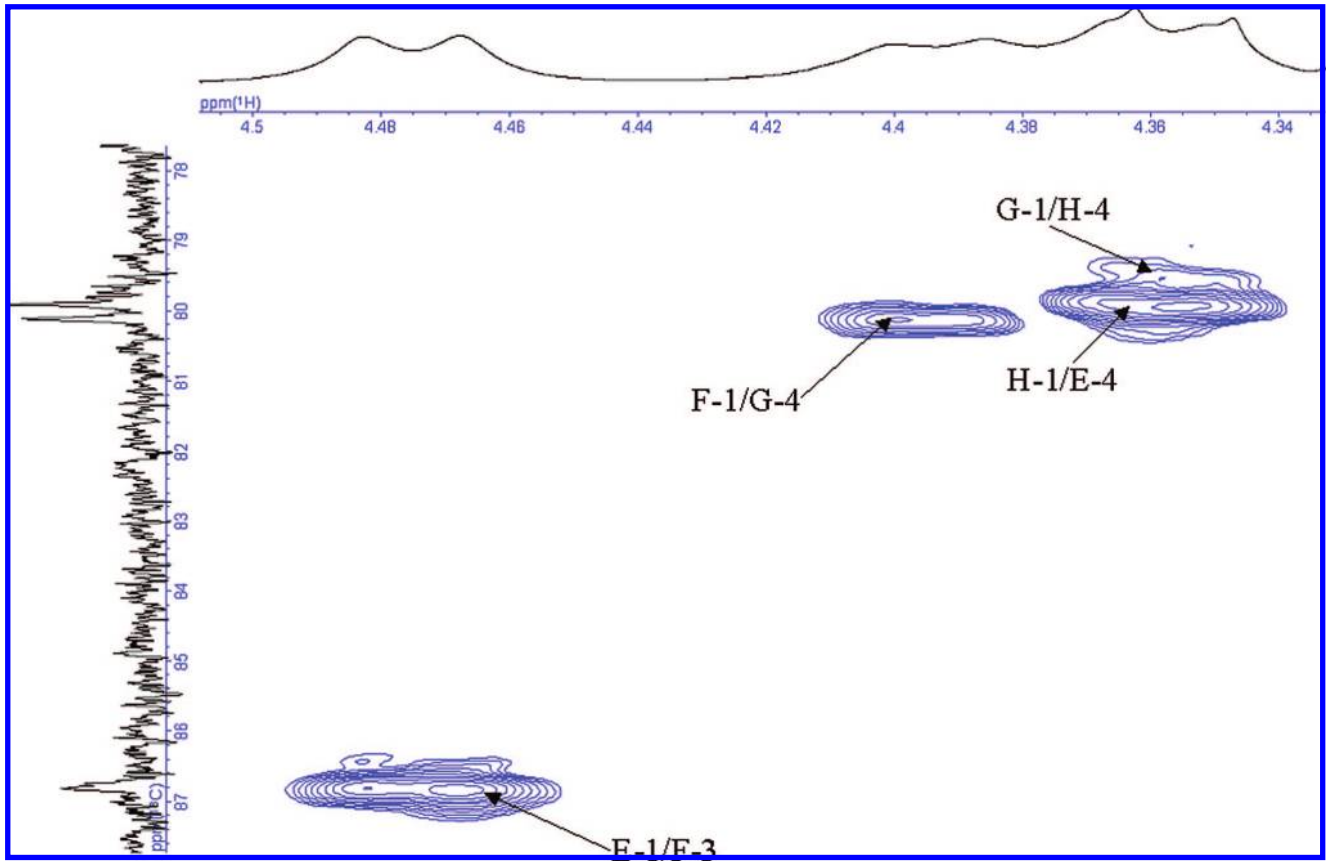

Figure 5. $^{1}H,^{13}C$ HMBC spectrum of barley β-glucan E70-S at 70 °C.

from the ^{1}H NMR signal integrations). We performed a 2D DOSY experiment to clarify whether the α-glucan moiety within BBG-70 is solely a contaminant or is covalently attached to the β-glucan moiety in order to analyze the difference between the diffusion coefficients of α-glucan and β-glucan. The signals derived from α- and β-glucan were well separated in the *F*2 dimension due to difference between their diffusion coefficients (**Figure 3**). This result indicated that the α-glucan moiety (residues A−D and I (possibly existing as a reducing terminal group)) and β-glucan moiety (residues E−H) were not covalently attached to one another, that is, the α-glucan moiety within the sample was merely a contaminant of BBG-70. This result can be explained by the fact that cereal barley is a starch-rich material. Therefore, we attempted to eliminate the α-glucan moiety within BBG-70 by the freeze–thawing method, as described under Materials and Methods, to confirm the assignments of residues and apply them in the subsequent biological test. These results were also confirmed by further purification of the material and assessment by 1D ^{1}H NMR spectroscopy (**Figure 1**, top).

Next, we attempted to assign all ^{1}H resonances in the ^{1}H NMR spectrum on the basis of COSY and TOCSY experiments. In the 2D TOCSY spectrum (**Figure 4**), a complete series of cross-peaks was observed between A H-1 and A H-2, 3, 4, 5, 6a, and 6b as well as between B, C, D, E, F, G, H, and I H-1 and between B, C, D, E, F, G, H, I H-2, 3, 4, 5, 6a, and 6b. However, some of their protons (i.e., between A H-5 and H-6a; between E H-3 and H-4 and H-5; between F H-2 and H-4; and between G H-3 and H-5) were ambiguous due to the extensive overlap of the neighboring ones. These ambiguously assigned protons were further studied using the overlaid spectra of ^{13}C-edited HSQC and H2BC to separate the neighboring protons in the ^{13}C dimension. That is, the overlaid HSQC and H2BC

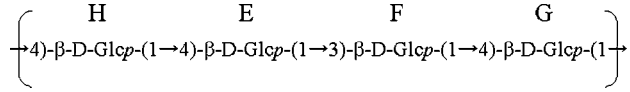

Figure 6. Representative structure of barley β-glucan E70-S: chemical structure of the β-glucans analyzed in this study. Glc*p*, glucopyranose.

spectra can trace out the entire intraring assignment as reported previously (*24, 27*). Thus, all protons in BBG-70 were assigned unambiguously.

In addition, the combination of the ^{13}C-edited HSQC and the HSQC-TOCSY spectra with various mixing times (30−120 ms) for TOCSY spinlock and the H2BC experiment allowed the complete assignment of the ^{13}C spectrum. **Table 3** summarizes the ^{1}H and ^{13}C NMR spectral assignments of BBG-70. These assignments are based on the COSY, TOCSY, HSQC, HSQC-TOCSY, and H2BC spectra.

In the spectrum of the β-glucan moiety within BBG-70, four cross-peaks (i.e., between H H-1 and E H-4, between E H-1 and F H-3, between F H-1 and G H-4, and between G H-1 and H H-4), which are shown in the ^{1}H, ^{13}C HMBC spectrum (**Figure 5**), were assigned to glycosidic linkages, as indicated by H(1 → 4)E, E(1 → 3)F, F(1 → 4)G, and G(1 → 4)H, respectively. These results were also confirmed by the NOESY experiment (data not shown). These data suggested a representative structure of pBBG-70, as described in **Figure 6**. As shown in this figure, the barley β-glucan (BBG-70) analyzed in this study is a linear mixed-linkage β-glucan composed of 1,3- and 1,4-β-D-glucopyranose polymers and contains discontinuous β-1,3-linked D-glucose residues.

Binding Capacity and Specificity to the *β*-1,3-Glucan Receptor, Dectin-1. Because the representative β-1,3-glucan receptor dectin-1 has been reported to play a significant role

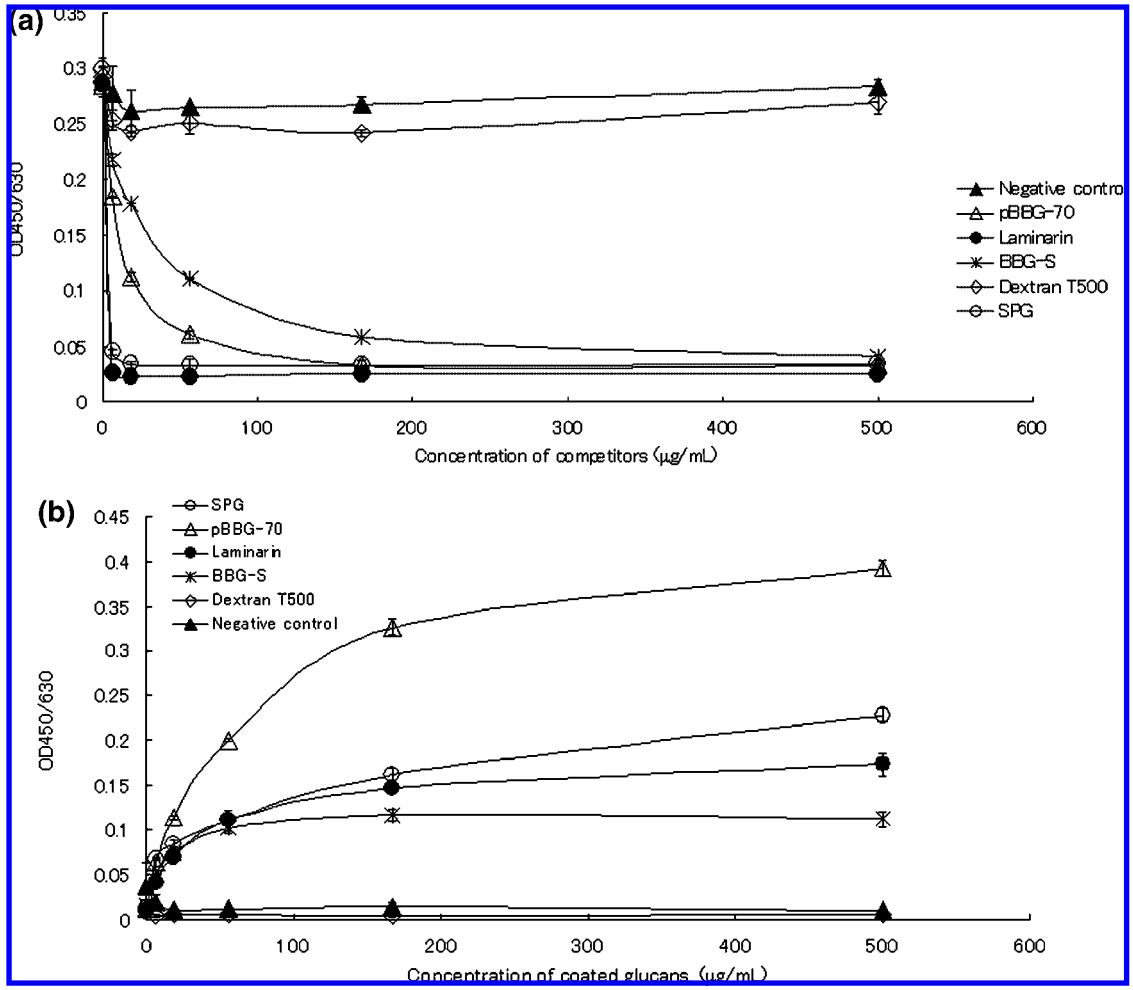

Figure 7. Binding specificity of barley β-glucan to the β-1,3-glucan receptor dectin-1. (**a**) The binding of barley β-glucan to dectin-1 was tested by competitive ELISA. An ELISA plate was coated with SPG. Various glucan samples, namely, (∗) BBG-S, (△) pBBG-70, (●) laminarin, (□) Dextran T-500, (○) SPG, and (▲) negative control, were diluted to achieve concentrations of 0−500 μg/mL and were mixed with biotin-labeled soluble dectin-1 (sDectin-1) before addition to the SPG-coated ELISA plate. The SPG-coated plate was first incubated with sDectin-1 and glucans and further incubated with peroxidase-conjugated streptavidin (Pharmingen). The binding of sDectin-1 to solid-phase SPG was monitored using the peroxidase substrate TMB and phosphate. (**b**) Reverse ELISA experiment was performed by coating the ELISA plate with various glucan samples at the indicated concentrations. Soluble dectin-1-Fc (sDectin-1-Fc) and peroxidase-conjugated antihuman IgG were added to the plate after blocking with 0.5% BSA/PBS. The absorbance of TMB was measured as mentioned above.

in some biological effects, we next investigated the binding capacity and specificity of pBBG-70 to recombinant sDectin-1. The competitive ELISA experiment revealed that pBBG-70 and BBG-S inhibited the binding of sDectin-1 to SPG, although at concentrations higher than those of SPG and laminarin (**Figure 7a**). On the other hand, dextran, which is a 1,3-α-glucopyranosyl-branched 1,6-α-glucan, remained unaffected. To confirm that the above-mentioned inhibitory action of pBBG-70 and BBG-S was not due to their nonspecific effect on dectin-1 binding, a reverse experiment was performed by coating the ELISA plate with various glucans and probing the sDectin-1 molecules on the solid phase of the glucans. Although dextran did not increase the absorbance when reacted with sDectin-1, the other glucan samples, including pBBG-70, SPG, laminarin, and BBG-S, significantly increased the absorbance when reacted with sDectin-1 in a dose-dependent manner (**Figure 7b**). It should be noted that pBBG-70 and BBG-S showed significant binding specificity for dectin-1. It is also interesting that barley β-glucan interferes with the dectin-1−SPG interaction, despite lacking the continuous β-1,3-D-glucosyl residues. Thus, the following speculations could be derived from these observations:

1. Dectin-1 recognizes only discontinuous β-1,3-linked D-glucose residues, although possessing a very weak affinity. Continuous β-1,3-linked D-glucose residues might be the only advantageous factor to compensate for the weak affinity to dectin-1.

2. Helical conformer, which is the characteristic conformation of β-1,3-glucans, might not be necessary for recognition by dectin-1.

3. 1,3-β- and 1,4-β-glucosyl structures within barley β-glucan can adopt the required conformation for binding to dectin-1.

4. The binding fashion to dectin-1 differs between the continuous β-1,3-linked D-glucose polymer, such as that in mushroom and yeast β-glucans, and the discontinuous β-1,3-linked D-glucose, such as that in barley β-glucan. However, further studies are required to reveal the precise mechanism(s).

These facts together with NMR spectroscopy results suggest that cereal barley β-glucan contains the ligand sugar

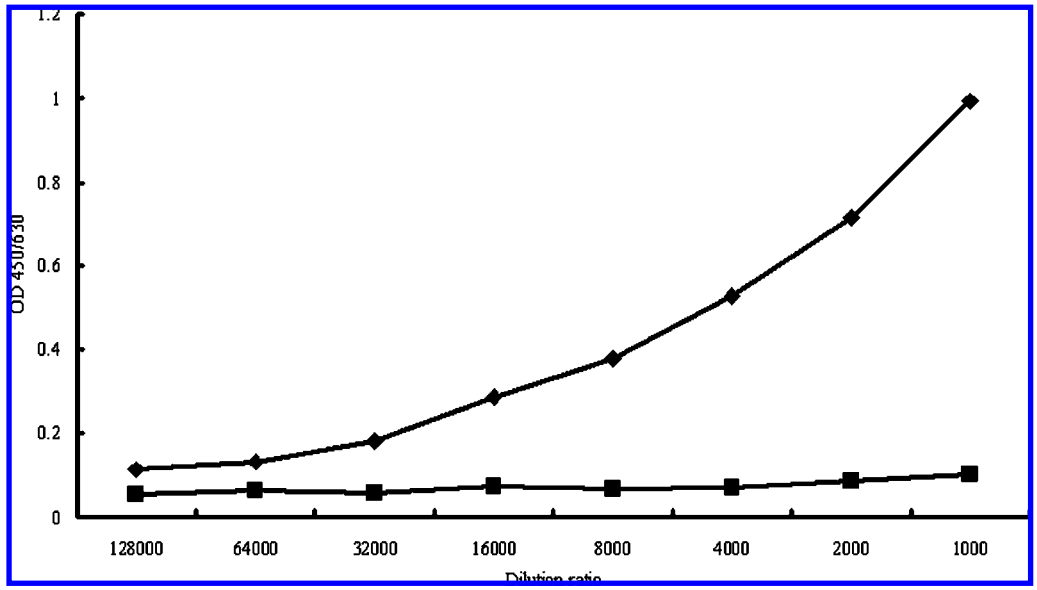

Figure 8. Comparison between the binding specificities of barley β-glucan and yeast β-glucan to Mab. The reactivities of pBBG-70 (■) and CSBG (□) to the Mab (1A5) were tested by ELISA.

structure that may be formed by 1,3-β- and 1,4-β-glucosyl linkage and that this structure is required for binding to the β-1,3-glucan receptor dectin-1.

Binding Capacity and Specificity to the Mab against Yeast β-Glucan. We recently developed a Mab against the *Candida* cell wall β-glucan, characterized this antibody as 1A5, and studied its reaction with a β-1,3-glucose polymer. We also used this Mab to further characterize pBBG-70. In this experiment, pBBG-70 did not react with the Mab yeast β-glucan (**Figure 8**). This result indicated the Mab against yeast β-glucan recognizes a distinct β-glucan epitope, which is also recognized by dectin-1; because pBBG-70 does not contain continuous β-1,3-D-glucose residues, which are essential for antibody binding, it did not react with the Mab.

In conclusion, numerous studies have been conducted on both the structural and biological characteristics of cereal barley β-glucans (*14–21*). However, currently, little information is available on their molecular mechanism(s). Our results showed that barley β-glucan, which is a linear mixed-linkage β-glucan composed of 1,3- and 1,4-β-D-glucopyranose polymers and contains discontinuous β-1,3-linked D-glucose residues, can be recognized by dectin-1, a pivotal receptor for β-1,3-glucan, but cannot bind to the Mab against yeast cell wall β-1,3-glucan. These findings implied that barley β-glucan might, at least in part, exhibit its biological effects via the recognition by dectin-1 of the ligand sugar structure, which may be formed by 1,3-β- and 1,4-β-glucosyl linkage. To our knowledge, the present study is the first to report this molecular mechanism of cereal barley β-glucan. The results of this study are useful not only in our understanding of the structure–activity relationship of β-glucans, including the structural requirements of dectin-1-binding ligands, but also in the promotion of the usage of a safer source of β-glucan than the bacterial and fungal derivatives, that is, barley β-glucan. This information regarding structural and ligand specificity can be used to shed light on molecular mechanism(s) such as β-glucan−dectin-1 interaction by NMR spectroscopy.

ABBREVIATIONS USED

BBG-S, barley β-glucan from Sigma; BBG-70, barley β-glucan E70-S; BRM, biological response modifier; CR3, complement receptor type 3; COSY, correlation spectroscopy; CRD, carbohydrate recognition domain; CSBG, β-glucan from *Candida albicans*; DB, degree of branching; DCs, dendritic cells; DOSY, diffusion-ordered spectroscopy; DP, degree of polymerization; ELISA, enzyme-linked immunosorbent assay; HMBC, heteronuclear multiple bond correlation spectroscopy; HSQC, heteronuclear single quantum correlation spectroscopy; H2BC, heteronuclear two-bond correlation spectroscopy; LacCer, lactosylceramide; Mab, monoclonal antibody; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser effect spectroscopy; pBBG-70, purified barley β-glucan E70-S; ROS, reactive oxygen species; SCG, β-1,3-glucan from *Sparassis crispa*; SPG, sonifilan; TOCSY, total correlation spectroscopy.

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