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The linear structure of β-glucan from baker's yeast and its activation of macrophage-like RAW264.7 cells

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ABSTRACT

Yeast β-glucan has many formulations with different chemical structures, water solubility and purity. In particular, the purity of β-glucan in these formulations is variable and relatively low, contributing to different data on its biological activity. In this study, the major polysaccharide component in the crude Baker's yeast polysaccharides coded as BBG with high purity of 99% was obtained, and its chemical structure was determined to be a linear β-(1,3)-glucan. It was found that BBG interacted with complement receptor 3 (CR3) and toll-like receptor 2 (TLR2) on the surface of macrophage-like RAW264.7 cells, and initiated activation of RAW264.7 cells characterized by significant production of tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein 1 (MCP-1). Additionally, activation of the nuclear factor kappaB p65 (NF-κB p65), c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) induced by BBG, were also observed, further confirming the stimulation of RAW264.7 cells by BBG. All these findings provided important scientific evidences for better understanding the molecular mechanism of action for the linear β-(1,3)-glucan in cells.

1. Introduction

Carbohydrates have long been underappreciated by the scientific community due to their multiple components and complex structures compared with proteins and nucleic acids, but they also have key roles in a broad range of biological processes, including transduction and immune responses. Therefore, studies of their structures and roles in cells are very important to clarify the mystery of polysaccharides in life-associated events. β-Glucan from yeast is a well-known biological response modifier functioning as an immunostimulant against infectious diseases and cancers through stimulation of granulocytes (neutrophils and eosinophils), monocytes, macrophages, and natural killer (NK) cells (Brown, Herre, Williams, Willment, Marshall, & Gordon, 2003; Hong et al., 2003; Hong et al., 2004; Li et al., 2006; Li et al., 2007; Salvador, Li, Hansen, Cramer, Kong, & Yan, 2008; Yan et al., 1999; Young, Ye, Frazer, Shi, & Castranova, 2001). Therefore, the roles of yeast polysaccharides attract much attention and have been extensively investigated in the world since it was first reported by Pillemer and Ecker (1941). For example, the crude yeast cell wall preparation (zymosan) is shown to be able to modulate non-specific immunity (Pillemer, Blum, Pensky, & Lepow, 1953), and the effective immune-activating compound in zymosan was identified to be β-glucan (Riggi and Di Luzio, 1961). Later, other formulations of yeast polysaccharides except zymosan including poly-(1,6)-β-D-glucopyranosyl-(1,3)-β-D-glucopyranose (PGG) with an average molecular weight of 170 ± 20 kDa (Patchen, Vaudrain, Correira, Martin, & Reese, 1998), a glucan sulfate (Williams et al., 1991), the

Abbreviations: TNF-α, tumor necrosis factor-α; MIP-2, macrophage inflammatory protein-2; IL, interleukin; MCP-1, monocyte chemotactic protein-1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; MAPK, mitogen-activated protein kinase; MAPKs, mitogen-activated protein kinases; JNK1/2, c-Jun N-terminal kinase 1/2; ERK1/2, extracellular signal-regulated kinases 1/2; MEK, mitogen-activated protein kinase, known as MAPK; IKK, inhibitor of NF-kappa B kinase; CR3, complement receptor 3; TLR2, toll-like receptor 2; NK cells, natural killer cells; PGG, poly-(1 → 6)-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranose; WGP, whole glucan particle; NSG, neutral soluble glucan; MyD88, myeloid differentiation primary response gene 88; CaMK, calmodulin (CaM)-dependent kinase; Pyk2, protein tyrosine kinase 2; CREB, cAMP response element-binding protein; PKC, protein kinase C; PLA2, phospholipase A2; PTK, protein tyrosine kinase; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; LPS, lipopolysaccharide; iNOS, inducible nitric oxide synthases; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; IFN, Interferon; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; PVDF, polyvinylidene difluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; BSA, bovine serum albumin; TBS, Tris-buffered saline; ECL, enhanced chemiluminescence.

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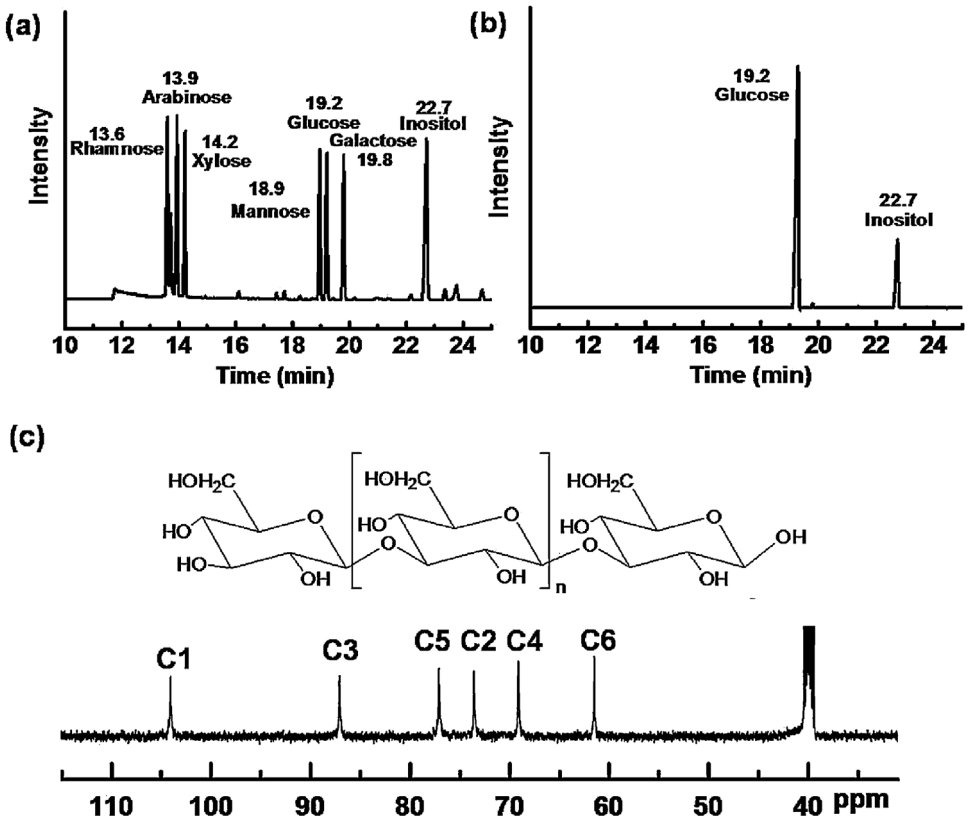

Fig. 1. The GC traces of monosaccharide standards (a) and hydrolyzed BBG (b), ^{13}C NMR spectra of BBG in DMSO-*d*6 at 298 K (c), and the repeating unit of BBG (inset).

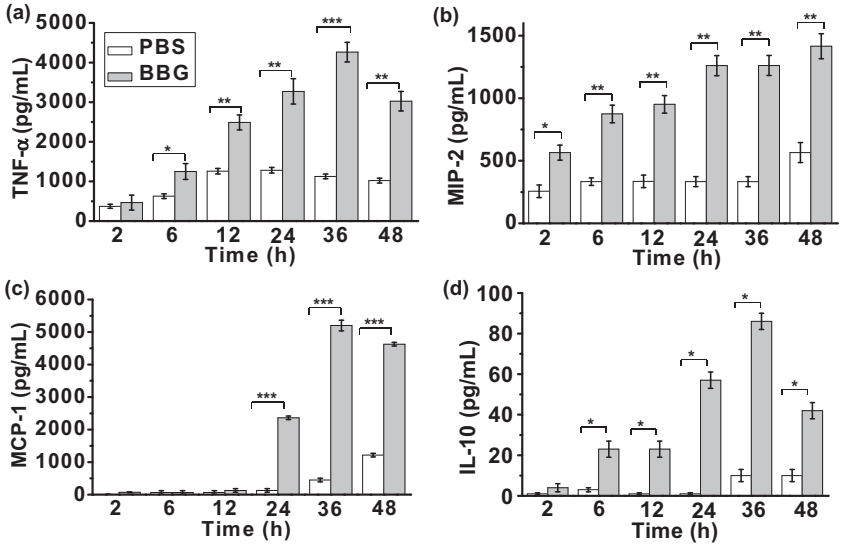

Fig. 2. Production of cytokines/chemokines in BBG-treated RAW264.7 cells. RAW264.7 cells (5×10^5 cells/well) were seeded into 24-well plates and incubated for 24 h before stimulation. At the end of pre-incubation period, cells were rinsed with PBS, and the cells were then exposed to BBG (200 μg/mL).After different time stimulation as indicated in the figure, the TNF-α (a), MIP-2 (b), MCP-1 (c) and IL-10 (d) levels in the supernatant were measured by ELISA kit as described under "Materials and Methods". Each value represents the mean ± SE of at least three separate experiments. Student's *t*-test was used to determine significant differences. $^*p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.001$ vs control (PBS).

neutral glucan with very small molecular weight (NSG, < 20 kDa) (Hong et al., 2003) and whole glucan particle (WGP, an insoluble particle) (Hong et al., 2004) were also reported. Of these, WGP and zymosan are water-insoluble (so also called particulate β-glucans), while PGG and NSG are water-soluble. Moreover, they are usually mixtures of different components or chemical structures. It has been shown that all these different formulations of yeast polysaccharides could exert biological activities. For instance, PGG may take part in decreasing the infectious complication rate in patients undergoing major surgery (Babineau et al., 1994), and WGP enhanced complement-mediated hematopoietic recovery after bone marrow injury (Cramer et al., 2006).

Many efforts have been made to understand the mechanism of action of how these glucans exert their biological effects (Adams,