BINDING OF FUNGAL AND PLANT GLUCANS TO THE HUMAN MACROPHAGE RECEPTOR

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Abstract

Glucans are α, β or α and β linked glucose polymers which may play a role in environmental inhalation toxicity. The mechanism(s) by which glucans act may involve binding to a human macrophage receptor. We examined the binding of five fungal (1→3)-β-D-glucans, a barley glucan and two non-β linked glucose polymers to the human macrophage cell line, U937. Competitive binding assays employed U937 (1 x 10^6), 15 µg of ^{3}H-(1→3)-β-D-glucan phosphate and increasing concentrations of unlabeled polysaccharides. Non-β-linked polymers did not bind. Scleroglucan, a branched (1→3)-β-D-glucan, showed a IC_{50}= 23 nM. Other glucans showed dissociation constants of 11 to 43 µM. Barley glucan exhibited a very low affinity binding K_D>50 µM. These data indicate that the glucan receptor is specific and that it has increased affinity for certain types of glucan.

Introduction

Glucans are polymers of glucose that are widely distributed throughout the biosphere (1). Specifically, glucans are found in the cell wall of plants, bacteria and fungi, as minor constituents of fungal cytosol and as polymers which are secreted into the environment by glucan producing microorganisms (1). Glucans can be broadly classified according to the type of intrachain linkage of the polymer, *i.e.* α- or β-linked (1). In addition, glucans are known to exist as branched or non-branched polymers (1,2). The β-linked glucans are the predominant form found in fungi (1). It is the fungal derived (1→3)-β-D-glucans which have been reported to modulate various aspects of macrophage activity and immunity (2-5). Recently, it has been proposed that glucans may play a role in the symptoms associated with sick building syndrome (6-15). This is due, in part, to their immunomodulatory activity and presence in the cell wall of fungi, which are frequently associated with "sick buildings".

The mechanism(s) by which glucans modulate immunity has not been defined. The first step in the modulation of macrophage activity by (1-3)-β-D-glucans is thought to involve binding to a specific receptor(16-18). We have reported receptor binding of a ^{3}H-glucan phosphate in both murine (J774a.1) and human (U937) macrophage cell lines (16). Similar results were obtained in both systems. In the human macrophage system, we observed a steep equilibrium (90 min) competition by unlabeled glucan phosphate with an observed rate constant of K_D = 37 µM, K_{ob} = 0.95min^{-1} and a B_{max} = 6.5 x 10^7 molecules per cell (16). The high number of binding sites more than accounts for the U937 cell surface area and consequently this data supports an uptake process (i.e internalization). We also demonstrated that pullulan a non-β-linked glucose polymer did not compete for binding (16). These data indicate the existence and specificity of the human macrophage (1-3)-β-D-glucan receptor.

The relationship between (1-3)-β-D-glucan structure and expression of immunobiologic activities has not been defined. This was due in part to the lack of well characterized (1-3)-β-D-glucan polymers with varying conformational structures. We have demonstrated that aqueous SEC/MALLS/DV can be employed to establish molecular mass moments, r.m.s. radii and polydispersity of water soluble (1-3)-β-D-glucan biological response modifiers (19-20). The purpose of this investigation was to compare and contrast the receptor binding affinity (K_D and B_{max}) of the various glucans in order to examine the effect of glucan polymer structure on receptor binding affinity.

Methods

Human cell line. We used the human promonocytic cell line U937. This cell line has been extensively utilized by our laboratory group in (1-3)-β-D-glucan receptor binding studies (16). U937 was maintained in RPMI-1640 medium with 10% serum protein supplement at 37ºC and 5% CO_2 tension.

Carbohydrate polymers. We evaluated eight water soluble carbohydrate polymers. Glucan phosphate and glucan sulfate were prepared from water insoluble (1→3)-β-D-glucan, isolated from *S. cerevisiae* as previously described (2,5). Schizophyllan (SPG, derived from *S. commune*) was obtained in sterile water (10 mg/ml) from Kaken Chemical Co. (Tokyo, Japan). Laminarin and Mannan were purchased from Sigma Chemical Co. (St. Louis, MO). Water soluble scleroglucan was prepared according to the protocol of Pretus et al. (21). Dextran was obtained from Pharmacia (Piscataway, NJ). Barley glucan was kindly provided by Dr. Peter Wood and Dr. Barry McCleary (Megazyme Ltd., Sydney, Australia). The primary structure of each carbohydrate polymer was confirmed by variable temperature FT-^{13}C-n.m.r. in $DMSO_{d6}$ at a concentration of 50 mg/ml as previously described (3). For the competitive displacement studies, stock solutions of the polysaccharides were prepared in RPMI 1640 cell culture media, filtered and subsequently diluted over a concentration range. The dn/dc values were determined with an Optilab 903 interferometric refractometer (Wyatt Technology, Santa Barbara CA) at 25ºC in 50 mM sodium nitrite mobile phase (16). To establish molecular mass and size, polydispersity, (weight-

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average molecular mass M_w/number-average molecular mass M_n) and intrinsic viscosity, the polysaccharides were analyzed by SEC/MALLS/DV as previously reported (19-20).

Receptor-binding assays. Water soluble (1 3)-β-D-glucan phosphate was radiolabeled as previously described by our group (16). Receptor binding was evaluated using the Millipore Multiscreen Assay System with 96-well-GF/C glass fibre filter plates (Millipore Corp., Bedford, MA). Displacement binding was determined in the present of a constant amount of radiolabeled ligand (15 µg/well) and increasing concentrations of unlabeled polysaccharide. After incubation at 37°C for 90 minutes the plates were vacuum filtered with subsequent washes (five times with warm serum free RPMI 1640) of the cells. The filters were then harvested and dried, and the radioactivity was determined by liquid scintillation counting (LSC 1409 Wallac Inc., Gaithersburg, MD) with a typical counting efficieny for tritiated glucan phosphate of 45% - 50%.

Data analysis. Binding displacement data for (1-3)-β-D-glucans were analyzed by nonlinear regression using models of one site competitive displacement (GraphPad Prism, San Diego, CA). Maximum binding was set to that seen in control (labeled glucan phosphate alone) preparations, and displacement set to 100% for polysaccharides without a measurable displacement plateau (*i.e.* glucan phosphate, glucan sulfate and schizophyllan). Polysaccharide concentrations displacing 50% of specific binding (IC_{50} values) were determined from the nonlinear regression fits.

Results

Competitive binding parameters for the carbohydrate polymers to U937. The IC_{50} values for the carbohydrate polymers are shown in Table 1. Scleroglucan exhibits the highest binding affinity to the human monocyte (1-3)-β-glucan receptor with a IC_{50} of 23 nM. This is approximately three orders of magnitude greater than the other carbohydrate polymers. The competitive binding affinities for the carbohydrate polymers was schizophyllan > laminarin > glucan sulfate > glucan phosphate (Table 1). We observed 100 % displacement of the labeled ligand with unlabeled schizphyllan and glucan phosphate. Scleroglucan, glucan sulfate and laminarin also displaced the labeled ligand, but at a lower level (Table 1). Dextran and mannan, which are non-β-linked glucose polymers, did not compete for binding with the radiolabeled glucan phosphate ligand. We observed a modest displacement of the labeled ligand with barley glucan at the highest concentrations. We speculate that this may be due to the increased viscosity of the barley glucan solutions.

Conclusions

Indirect evidence continues to mount concerning the potential involvement of (1→3)-β-D-glucans in the symptoms associated with sick building syndrome. The cellular and molecular mechanisms by which (1→3)-β-D-glucans modulate immunity are just beginning to emerge. We (2-5) have shown that glucans mediate immunological activity, in part, via macrophage participation. Fogelmark *et al.* (15) have speculated that glucans mediate pulmonary effects due, in part, to their effect on macrophages. The first step in the interaction of glucan with mammalian macrophages is thought to involve the binding of (1→3)-β-D-glucan to a receptor (16-18). Data from our laboratory employing the human promonocytic cell line, U937, indicates that glucan phosphate binding obeys the criterion for specific binding in that competition for the binding sites can be demonstrated in the presence of a 10- fold excess of unlabeled ligand (16). We have examined U937 cells by electron microscopy following glucan exposure and observed an increase in phagolysosome formation which is consistent with uptake of glucan (16). We concluded that the binding and internalization of glucans by human macrophages is a two-phase process; the first phase is a rapid binding of the glucan ligand to the receptor followed by a slower uptake/internalization phase.

The present data confirm and extend our initial observations by demonstrating the specificity of the human macrophage glucan receptor for (1→3)-β-D-glucans. More importantly, these data indicate that the glucan receptor has a higher affinity for one form of glucan over another, *i.e.* branched versus non-branched.We also observed that there is a dramatic difference in the affinity of the receptor among branched glucans. Scleroglucan shows a IC_{50} of 23 nM which is three orders of magnitude greater than any of the other glucans. Scleroglucan is a (1→3)-β-D-glucan with (1→6)-β-linked side chain branches occurring on average every third subunit along the polymer backbone (21). Schizophyllan (SPG) is also a branched (1→3)-β-D-glucan. The side chain branches of SPG occur at approximately the same frequency as those observed for scleroglucan. Thus, there must be other differences between these two highly branched polymers that can account for the difference in receptor affinity. Additional studies will be required in order to elucidate the precise nature of these differences. These studies also documented that a mixed linkage glucan isolated from barley did not effectively compete for binding. We speculate that this may be due to the dramatic increases in specific viscosity which occur as the concentration of barley glucan is increased. We conclude that the human macrophage (1→3)-β-D-glucan receptor is specific for (1→3)-β-D-glucans. Of potentially greater importance, the receptor can discriminate between glucans as denoted by differences in affinity. Whether these differences in binding affinity correspond to differential expression of biologic function remains to be determined.

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Table I. Competition binding (IC_{50}) of fungal and plant (1→3)-β- D-glucans to the U937 promonocytic cell line.

Glucan	IC_{50} (μM)	Displacement (%)
Glucan phosphate	43	100
Glucan sulfate	35	37
Barley glucan	85	100
Scleroglucan	0.023	40
Schizophyllan (SPG)	11	100
Laminarin	21	57