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Structure–immunomodulatory activity relationships of dietary polysaccharides

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ABSTRACT

Polysaccharides are usually composed of more than ten monosaccharide units, which are connected by linear or branched glycosidic bonds. The immunomodulatory effect of natural polysaccharides is one of the most important bioactive function. In this review, molecular weight, monosaccharide (including galactose, mannose, rhamnogalacturonan-I arabinogalactan and uronic acid), functional groups (namely sulfate, selenium, and acetyl groups), types of glycoside bond connection (including β-1,3-D-glucosyl, α-1,4-D-glucosyl, β-1,4-D-glucosyl, α-1,6-D-glucosyl, β-1,4-D-mannosyl, and β-1,4-D-Xylopyranosyl), conformation and the branching degrees are systematically identified as their contribution to the immunostimulatory activity of polysaccharides. At present, studies on the structure-activity relationships of polysaccharides are limited due to their low purity and high heterogeneity. However, it is an important step in providing useful guidance for dietary supplements with polysaccharides. The chemical structures and the process of immune responses induced are necessary to be discussed. Polysaccharides may bind with the cell surface receptors to modulate immune responses. This review mainly discusses the structure-activity relationship of dietary polysaccharides.

1. Introduction

Dietary polysaccharides are widely distributed in plants, animals, and microbes. Natural polysaccharides have attracted research interest due to their unique nutritional value. Their various health promotion functions are designed to show the anti-dabetic, anti-viral, anti-inflammatory, and immunomodulatory effects (Tang et al., 2019; Yu et al., 2022; Zhao et al., 2020a, 2020b; Zhang et al., 2021d; Zong et al., 2012). Polysaccharides, as the dietary nutritional components, are absorbed by the small intestine and then enhance the innate and adaptive immune response of the host. The activation of macrophages is an important part of innate and adaptive immunity (Yu et al., 2012). Upon recognition of specific receptors on the surface of macrophages by polysaccharides, the relevant signaling pathways are activated to result in the release of bioactive molecules and inflammatory cytokines (Lei et al., 2015; Lu et al., 2017). Inhibition of tumor growth or enhancement of immune function of the intestinal system is another possible immunological effect of polysaccharides (La Fata et al., 2018). Galectin plays an important role in inflammatory response and tumor metastasis. It is reported that the intracellular polysaccharides (IPS) and extracellular polysaccharides (EPS) from *Penicillium oxalicum*can can reduce the risk of tumor formation and enhance immune activity by inhibiting galectin (Zhang et al., 2021a). Polysaccharides enhance human immunity by activating different immune-pathways. As the natural defense mechanism of the body, immunity plays a great role in fighting infectious diseases as well as regulating inflammation. People with low immunity are prone to various infections and tumors because low immune function weaken immune surveillance.

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The molecular weight is an important feature in the structure-activity relationship of polysaccharides. Some particular monosaccharides, such as galactose, mannose, rhamnogalacturonan-I and arabinogalactan or uronic acid are closely associated with immune-enhancement. The chemical modification of polysaccharides can promote the biological activity of polysaccharides, and even create new activities (Xu et al., 2019). The addition of sulfate, selenium, and acetyl groups to the polysaccharides modifies their structures to easily enter the cells of the immune system and hence may trigger different immune stimulation responses (Lu et al., 2021; Ren et al., 2007; Zhan et al., 2022). Polysaccharides have different conformations, both triple-helix and random coil have their own characteristics and can be recognized by the receptors of immune cells. Polysaccharides with immune-enhancing effect can have the straight chains or branching chains connected to the main chain. The chain branching affects solubility, molecular weight and chain conformation of the polysaccharides, leading to the boost of interaction between polysaccharides and cells, thus affecting the immune activity (Mueller et al., 2000).

Many polysaccharides have been shown to stimulate the immune system through different pathways, but there is still a lack of research on their structure-activity relationship. In this review, the similarities and differences of polysaccharides in molecular weight, monosaccharide composition, chemical modification, glycosidic bond composition, chain conformation and branching degrees are discussed, in order to navigate the structure-activity relationship of polysaccharides and their contribution to the immune system.

2. Effect of molecular weights of polysaccharides on immune activity

Studies have shown that the biological activity of polysaccharides is closely related to molecular weight (Zong et al., 2012). The molecular weight of *Laminaria* polysaccharide with immune activity is between 6 and 8 kDa (Elyakova et al., 2007). Polysaccharides with high-molecular-weight extracted from seven herbs (*Lentinula edodes*, *Ganodorma lucidum*, *Tremella fuciformis*, *Chrysanthemum*, *Lycium barbarum*, *Codonopsis pilosula*, and *Poria cocos*) have the better effect on macrophages, especially when the molecular weight is between 100 and 1000 kDa. The polysaccharide fraction with molecular weight between 100 and 1000 kDa showed the strong activity by directly stimulating NO release and the secretion of cytokines by macrophages (Deng et al., 2020). Two polysaccharide components F1 and F2 are extracted from *Chlorella ellipsoidea*, with molecular weights of 126.9 kDa and 237 kDa, respectively (Qi and Kim, 2017). The F2 with high-molecular-weight exhibited its higher NO-releasing capacity. Studies have shown that molecular weight is a key factor affecting the immune activity of *C. ellipsoidea* polysaccharides (Qi and Kim, 2018). Okra polysaccharides with homogalacturonan have the immune modulating activity on macrophages to cross-link with Toll-like receptors as related to molecular weight (Leung et al., 2004; Vogt et al., 2016). Treatment of macrophages with okra polysaccharides led to increased expression of interleukin-8 (IL-8), IL-1β, and tumor necrosis factor-α (TNF-α) (Trakoolpolpruek et al., 2019). The molecular weights of two polysaccharide components extracted from the dried *Coriolus versicolor* fruiting bodies are 29.7 kDa and 50.8 kDa, respectively. Among them, the high-molecular-weight component can promote a better release of NO and TNF-α expression (Zhang et al., 2021b). *Gentiana crassicaulis* root polysaccharides with high-molecular-weight have the strong effect of complement activation (Zou et al., 2017). However, carrageenan from the red alga *Solieria chordalis* with low molecular weight (<20 kDa) and lentinan polysaccharides with medium molecular weight between (10–10000 kDa) also have higher immune activity (Stephanie et al., 2010; Zhang et al., 2005). *Lycium barbarum* polysaccharide A4 with molecular weight of 10.2 kDa showed anticancer activity, while P8 with 6.5×10^3 kDa has non activity (Zhang et al., 2013). Low molecular weight oat β-glucans significantly reduced the survival rate of cancer cells, while they are not toxic to normal cells. High molecular weight oat β-glucans showed lower immunity due to their high viscosity (Choromanska et al., 2015). With appropriate molecular weight, β-glucans will have a more extended chain conformation and show a stronger affinity for receptors on the cell surface, resulting in higher immune activity (Ping et al., 2016). The extracellular polysaccharide fraction isolated from *Porphyridium cruentum* with a molecular weight of 6.53 kDa had the strongest immune-enhancing activity, as evaluated by the S180-tumor-bearing mouse model *in vivo* and peritoneal macrophage activation *in vitro* (Sun et al., 2012). The inhibition of *Dendrobium huoshanense* stem polysaccharides on gastric cancer *in vivo* was closely related to the molecular weight. With the decrease of the molecular weight, their anti-gastric cancer activities exhibited a decreasing trend (Liu et al., 2021). in contrast, low-molecular-weight polysaccharide GCP-2 from *Chaetomium globosum* CGMCC 6882 presents the inhibitory effects on *Escherichia coli* and *Staphylococcus aureus* than high-molecular-weight one GCP-1. They possessed immune activity by influencing the cell membrane integrity, Ca^{2+}-Mg^{2+}-ATPase activity at the cell membrane, and calcium ions in the cytoplasm of *E. coli* and *S. aureus* (Zhang et al., 2021a).

In support, many studies have suggested that the immunostimulating activities of polysaccharides are related to their molecular weights. In particular, polysaccharides with molecular weights between 10 and 1000 kDa showed better immune activities in numerous researches, while less than 10 kDa or more than 1000 kDa ones exhibited the weak immunomodulatory activity. Furthermore, polysaccharides with a molecular weight of >1000 kDa affect the diffusion and absorption, which are not conducive to across the membrane into immune cells. Meanwhile, polysaccharides with a molecular weight of <10 kDa can not maintain the chain conformation and exhibit good activity. It was found that the appropriate molecular weight is helpful to improving biological activity because of the increased number of sulfate groups in the broken chains (Qi et al., 2005). Above all, molecular weight is considered to be a significant structural feature of structure-function relationships. The molecular weights of the polysaccharides above are shown in Table 1.

3. Effect of monosaccharide domain of polysaccharides on immune activity

3.1. Effect of monosaccharide compositions on immune activity

Two polysaccharides CAVAP-I and CAVAP-II from *Citrus aurantium* Linn. Variant *amara* Engl showed significantly immunostimulatory activity via the promotion of IL-6, TNF-α, and IL-1β. Monosaccharide composition analysis revealed that CAVAP-I and CAVAP-II mainly contained arabinose, mannose, glucose, and galactose with different ratios, which might be the reason why CAVAP-II has a different immune-enhancing potential than CAVAP-I (Shen et al., 2017). With relatively high ratios of arabinose, galactose, xylose, and uronic acid, *Helicteres angustifolia* L. polysaccharide could significantly enhance the proliferation of macrophages, and stimulate the macrophages phagocytic capacity, as well as induce NO and immunomodulatory cytokines (Sun et al., 2019). Using multiple linear regression analysis, correlations between the compositions of arabinose, galactose, xylose, and mannose, and macrophage stimulatory activities *in vitro* were revealed (Lo et al., 2007; Sun et al., 2015).

3.2. Effect of high galactose content on immune activity

Ginkgo biloba polysaccharides rich in galactose (Gal) have better immunomodulatory activity by promoting NO and cytokines for macrophages (Fang et al., 2020; Ren et al., 2019). *Gracilaria lemaneiformis* polysaccharides rich in galactose at 45.84%, have good immunomodulatory effect via increasing its pinocytic ability to macrophages in a dose-dependent manner (Ren et al., 2017). Pectin from the bee pollen of *Nelumbo nucifera* contained rhamnose (11.5%), galactic acid (12.0%),

Table 1
The molecular weight of different polysaccharide species and their immunological activity.

Sources	Compound	Molecular weight	Mechanisms	References
Herbs	CD1	>1000 kDa	Stimulate NO release and induce macrophages to secrete cytokines	Deng et al. (2020)
	CD2	100–1000 kDa		
	CD3	10–100 kDa		
	CD4	<10 kDa		
Chlorella ellipsoidea	F1	126.9 k Da	Stimulate macrophages to produce considerable amounts of nitric oxide and various cytokines	Qi and Kim (2017)
	F2	237 kDa		
Okra	OP	>640 kDa	Increase IL-8, IL-1β and TNF-α expression	Trakoolpolpruek et al. (2019)
Coriolus versicolor	CVPn	29.7 kDa	Promote the release of NO and TNF-α mRNA expression	Zhang et al. (2021c)
	CVPa	50.8 kDa		
Gentiana crassicaulis roots	GCP-I-I	627.3 kDa	Exhibit potent complement fixation	Zou et al. (2017)
	GCP–II–I	471.3 kDa		
Carrageenan		<20 kDa	Enhance neutrophil phagocytosis and stimulate lymphocyte proliferation	Stephanie et al. (2010)
Lycium barbarum	lbp-a4	10.2 kDa	Immunity by inhibiting the proliferation of cancer cells	Zhang et al. (2013)
	lbp-p8	6.5×10^3 kDa		
Oat	β-glucan		Immunity by decreasing cancer cell viability	Choromanska et al. (2015)
Porphyridium cruentum		6.53 kDa	Stimulate the ability of macrophages to proliferate	Sun et al. (2012)
Dendrobium huoshanense stem			Inhibit tumor angiogenesis and enhance T cell immune response to induce tumor cell apoptosis.	Liu et al. (2021)
Chaetomium globosum	GCP-1	5.340×10^4 Da	Inhibitory effects against *Escherichia coli* and *Staphylococcus aureus*	Zhang et al. (2021a)
	GCP-2	3.105×10^4 Da		

galactose (41.2%), and arabinose (29.7%), especially. And it exerts its immunomodulatory ability by promoting the production of NO, lymphocyte proliferation, and macrophage phagocytosis. Galactose side-chain residues might play an important role in macrophage phagocytosis (Li et al., 2018b). Based on the structure-activity relationship of the above polysaccharides rich in galactose, they have a good response when its content reaches about 40% of total.

3.3. Effect of high mannose content on immune activity

Passiflora foetida polysaccharides with mannose accounting for 48.83%, could promote the secretion of NO, TNF-α, and IL-6 by macrophages (Song et al., 2019a). Previous studies found that *Auricularia auricula-judae* polysaccharides had specific repeating units, including mannose, glucuronic acid, xylose, trace galactose, and glucose. It promotes the secretion of proinflammatory cytokines by macrophages through activation of the TLR4 signaling pathway when the ratio of mannose was as high to 65%. The molecular docking of *A. auricula-judae* polysaccharides with TLR4 is shown in Fig. 1A (Perera et al., 2018). *A. auricula-judae* polysaccharides contain many sugar rings and active groups, which form hydrogen bonding interactions with the active sites of TLR4/MD2 protein (GLN-80, LYS-56, ASN-57, GLN-38, ASN-70, ASP-141, and LYS-152). It effectively stabilizes *A. auricula-judae* polysaccharides on the surface or active pocket of the protein, and promote the formation of polysaccharide-TLR4 complex. *Dendrobium devonianum* polysaccharide richs in β-1,4-D-mannosyl, which might contribute to the binding of mannose receptors on macrophages to induce activation of immune (Deng et al., 2018). *Craterellus cornucopioides* polysaccharides could improve the proliferation activity of macrophages in a certain range of concentrations and periods. Monosaccharide composition analysis revealed that *C. cornucopioides* polysaccharide contained mannose, galactose, glucose, and xylose, which mannose can reach 48.73% (Guo et al., 2019). *Aloe vera* polysaccharide contained mannose enhances the activity of splenic lymphocytes, macrophages and dendritic cells (Liu et al., 2019). Another study also has shown that this kind of polysaccharide enhances the lymphocyte response to alloantigen, which may be related to the release of IL-1 (Ferreira et al., 2015). Polysaccharides with high mannose had better immune-enhancing activity probably because they were easily recognized by receptors (Figueiredo et al., 2012). The perspective of previous studies suggested that about 50% or more than 60% mannose content account for the interaction between polysaccharide and receptor on the immune cells.

3.4. Effect of high rhamnogalacturonan-I content on immune activity

Molokhia leaf polysaccharide had good prebiotic and intestinal immune enhancement activity, which can improve the proliferating activity of bone marrow cells and promote the production of immunoglobulin A and cytokines. The content of rhamnose (22.4%) and galacturonic acid (22.1%) was high in Molokhia leaf polysaccharide, indicating that it has rhamnogalacturonan I (RG-I), which is the active part of molokhia leaf polysaccharide (Lee et al., 2021). The association with homogalacturonan and RG-I is an important structural feature of okra polysaccharides that stimulate macrophage proliferation (Trakoolpolpruek et al., 2019). Both pectic polysaccharides from *Prunus avium* significantly induced the NO release and the expression of several immune-related cytokines in macrophage cells. Structural analysis indicated that both fractions were RG-I pectic polysaccharides with glycan side chains (Cao et al., 2018). Above all, RG-I is one special functional monosaccharide domain, showing high rhamnose and galacturonic acid in the immune polysaccharide.

3.5. Effect of high arabinogalactan content on immune activity

Arabinogalactan (AG) is a highly branched neutral monosaccharide domain composed of arabinose and galactose. GCP-I-I and GCP–II–I are polysaccharides extracted from *Gentiana crassicaulis* roots with a strong complement activation effect. The complement system is one of the significant parts of the innate immune system, cooperating with the adaptive immune system (Dunkelberger and Song, 2010). A higher amount of arabinogalactan type I (AG-I) and arabinogalactan type II structures (AG-II) present in fraction *Gentiana crassicaulis* root polysaccharides I than that in fraction *G. crassicaulis* polysaccharides II, may explain the higher complement fixation activity of fraction *G. crassicaulis* polysaccharides I (Zou et al., 2017). *Parkia biglobosa* bark polysaccharide which is a pectin polysaccharide containing AG-II shows great activity in complement binding tests and can promote macrophages to secrete NO (Zou et al., 2014). Based on a comparison of the different fractions, researches showed that AG-I and AG-II in *Lessertia frutescens* leaf polysaccharide were important for its immune activity (Zhang et al., 2014). *Ixeris polycephala* polysaccharide, an arabinogalactan, which increased phagocytosis of macrophages and enhanced the production of NO was mainly composed of arabinose and galactose in a molar ratio of 28.1% and 70.3% respectively (Luo et al., 2018). In a word, being rich in arabinogalactan means combining a high amount of arabinose and

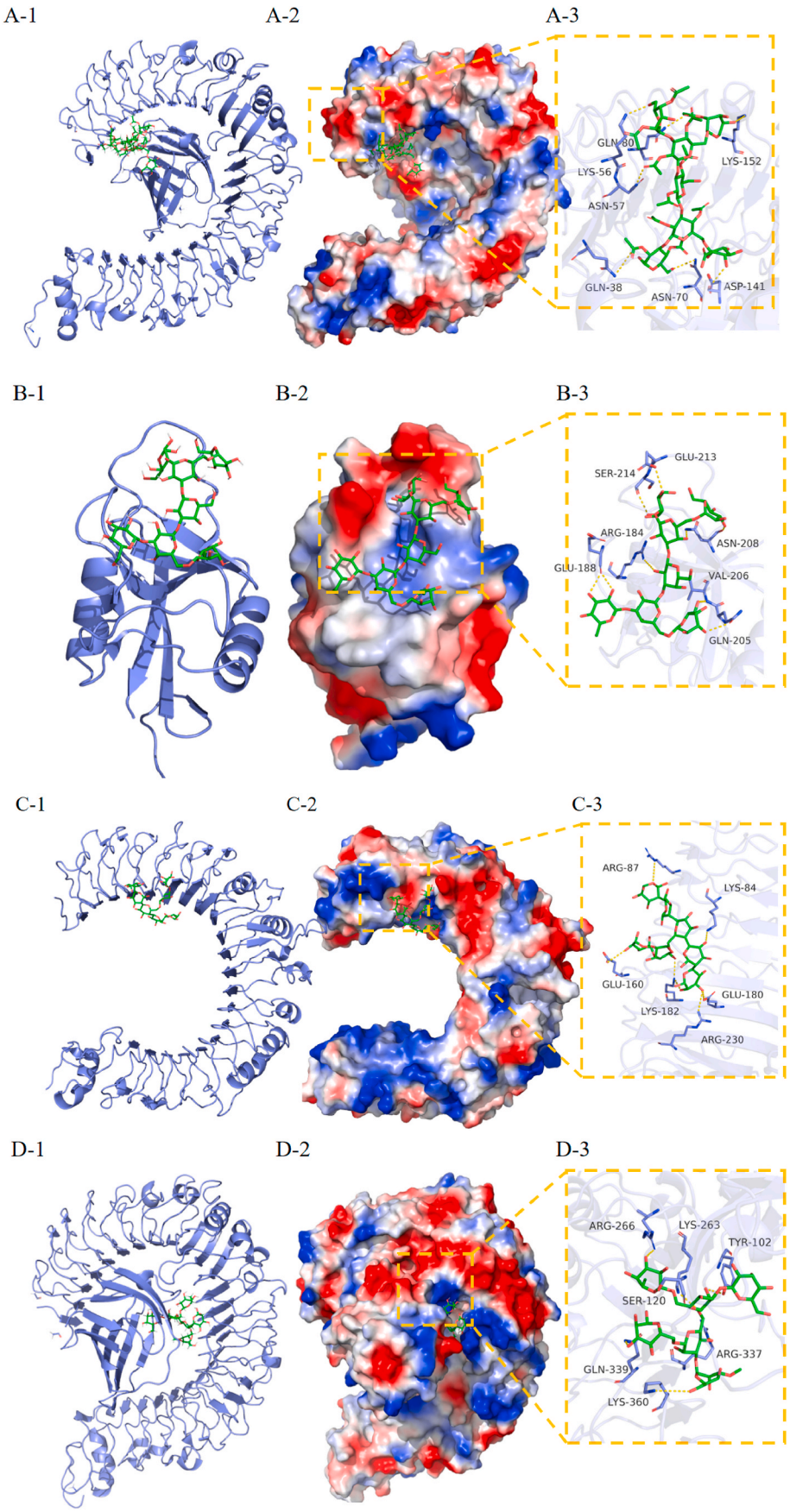

Fig. 1. The molecular docking of polysaccharides with immune cell receptors. A1, *A.auricula-judae* polysaccharide-TLR4 compound; A2, the electrostatic surface of TLR4; A3, the combination mode between *A.auricula-judae* polysaccharides and TLR4; B1, *P. cicadae* polysaccharide-TLR4 compound; B2, the electrostatic surface of TLR4; B3, the combination mode between *P. cicadae* polysaccharides and TLR4; C1, lentinan polysaccharide-Dectin-1 compound; C2, the electrostatic surface of Dectin-1; C3, the combination mode between lentinan polysaccharides and Dectin-1; D1, *P. boydii* polysaccharide-TLR2 compound; D2, the electrostatic surface of TLR2; D3, the combination mode between *P. boydii* polysaccharides and TLR2.

galactose, therefore exhibiting a strong-immune response due to the formation of a particular conformation.

3.6. Effect of high uronic acid content on immune activity

Two purified polysaccharides named WPMP-1 and WPMP-2 were obtained from *Polygonum multiflorum* with activation effects on splenocytes and macrophages. However, WPMP-2 shows better bioactivities than WPMP-1, which indicated that higher content of uronic acid may be associated the immunomodulatory activity (Zhang et al., 2018). The pomegranate peel polysaccharides induced murine macrophage cells to release a considerable number of inflammatory mediators. The content of uronic acids in it reached 19.9%–30.8% (Ahmadi Gavlighi et al., 2018). *Hovenia dulcis* peduncles polysaccharide with 45.9% of uronic acid content had an activation effect on macrophages. The results showed that the uronic acid played important role in immune activity *in vitro* (Wang et al., 2017). Consequently, it is summarized that the proper ratio of uronic acid to enhance immunity is between 20% and 50%.

Above all, polysaccharides containing monosaccharide domains like galactose, mannose, rhamnogalacturonan-I, arabinogalactan, and uronic acid may have immunostimulatory activity. Various forms of monosaccharides significantly suppress immune factors and especially impact the changes on downstream adaptive immune system, which can use the immobilized monosaccharides with immune-regulatory polysaccharides to investigate the modulation of immunity (Alobaid et al., 2020). Due to specific monosaccharide receptors on the immune cell membrane, the polysaccharide containing monosaccharide domains mentioned above may trigger diverse signal pathways and then lead to detectable immune responses. Their specific mechanisms are shown in Fig. 2.

4. Effect of chemical modification of polysaccharides on immune activity

4.1. Effect of the sulfation of polysaccharides on immunomodulation

Sulfation lentinan has stronger immune activity, and can improve the human immune response to the Newcastle disease vaccine by increasing the number of antibodies and leukocytes (Guo et al., 2009). Yam polysaccharide and sulfated yam polysaccharide have the same monosaccharide, however, sulfated yam polysaccharide could increase the immunomodulatory activity on splenic lymphocytes by inducing splenic lymphocytes differentiation into T lymphocytes (Huang et al., 2020). The immunomodulatory effect of sulfated *Ganoderma atrum* polysaccharides is closely related to the degrees of substitution (DS) of their sulfate groups. *G. atrum* polysaccharides with moderate DS and medium molecular weight exhibit the highest immunomodulatory activity by increasing the macrophage phagocytosis capacity and TNF-α production (Chen et al., 2015). The sulfated polysaccharides extracted from red algae were found to possess the activity of inhibiting tumor cells, while the original ones do not (Bürgermeister et al., 2002). Sulfated Chinese date polysaccharides had stronger immune activity, prolonging partial thrombin time, but did not change the prothrombin time (Li and Huang, 2021). In the study of sulfated polysaccharides from *Ulva rigida* C. Agardh, it was concluded that the level of NO released from macrophages was proportionally related to the DS of sulfate groups (Leiro et al., 2007). The sulfate groups of sulfated *Citrus medica* L. var. sarcodactylis polysaccharide may enhance the immunoregulatory

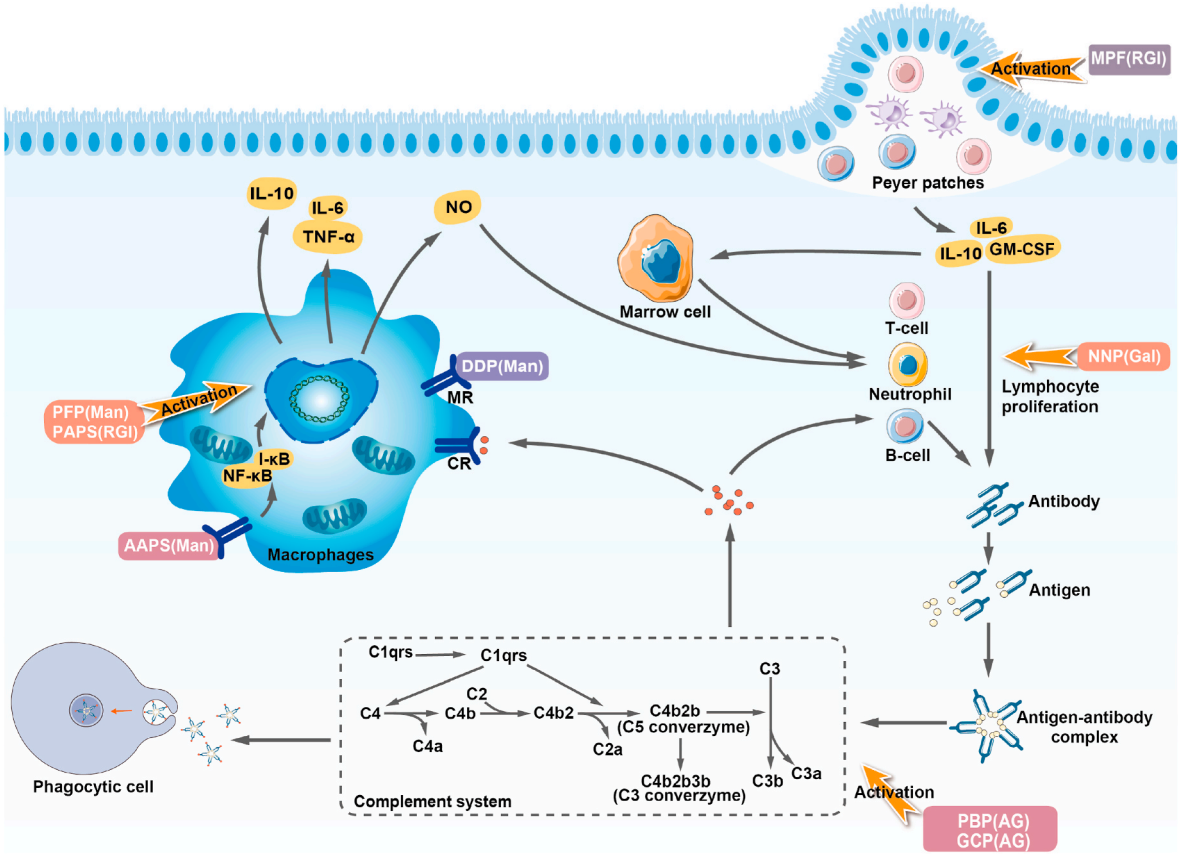

Fig. 2. Regulatory mechanisms for activation by polysaccharides with immune-enhancing effects. Abbreviations: NNP, *Nelumbo nucifera* polysaccharide; PFP, *Passiflora foetida* polysaccharide; AAPS, *A. auricula-judae* polysaccharides; DDP, *Dendrobium devonianum* polysaccharide; MPF, Molokhia leaf polysaccharide; PAPS, *Prunus avium* polysaccharides; GCP, *Gentiana crassicaulis* polysaccharide; PBP, *Parkia biglobosa* bark polysaccharide. The monosaccharide components contained in the immunostimulatory polysaccharides are in brackets.

activity by twisting and converting sugar ring configuration and orientation, exposing the hydroxyl groups, thus improving the interaction between the sulfated polysaccharide and specific receptors (Peng et al., 2019). The content of sulfate and the mode of sulfation have an important impact on the immunological activity of sulfated polysaccharides (Caputo et al., 2019). In short, the ideal DS of sulfation ranges from 1.5 to 2 according to a large number of studies (Lu et al., 2021). After sulfation on the polysaccharide, the sulfated hydroxyl groups change in electrostatic repulsion and steric hindrance. The negatively charged sulfate groups in sulfated polysaccharides can interact with the positively charged amino acids in the virus surface glycoprotein to block the binding sites where the virus binds to the host cell receptors (Lu et al., 2021).

4.2. Effect of the selenylation of polysaccharides on immunomodulation

The selenization of polysaccharides is of great importance to evaluate the effect of immunomodulatory activity. After selenylation of lily polysaccharide can promote lymphocyte proliferation and enhance the serum antibody titers and IL-6 contents more significantly (Hou et al., 2016). *Sagittaria sagittifolia* L. polysaccharides modified with selenium were shown to have higher immunomodulatory activity than the unmodified polysaccharides (Feng et al., 2022). *In vitro* experience, compared with the original polysaccharide, selenized *Codonopsis pilosula* polysaccharide better promoted the proliferation of lymphocytes and increased the proportion of T cells. *In vivo*, selenized *Codonopsis pilosula* polysaccharide also dramatically increased the level of immune factors, suggesting that selenylation can improve immune activity (Gao et al., 2020). Selenylated exopolysaccharides (Se-EPS) from *Lactococcus lactis* subsp. *lactis* have better immune-enhancing activity than original polysaccharides, as reflected by hemolytic complement activity. Se-EPS significantly enhanced the phagocytosis of peritoneal macrophages, as well as increased spleen and thymus indices, suggesting that they enhanced the immune function of the organism by modulating non-specific cellular and humoral immunity (Guo et al., 2013). Selenized *Artemisia sphaerocephala* polysaccharides with high selenium content exhibited stronger immunomodulatory activity by upregulating the phosphorylation levels of extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and phosphorylated 38 (p38), thus enhancing the proliferation and phagocytosis of macrophage cells (Li et al., 2020). In general, the content of selenium is widely used to increase the effect of immunomodulatory activity in polysaccharides, especially selenium content of about 40 $mg{\cdot}g^{-1}$ (Hou et al., 2016). After selenylation, the strong attraction between selenium surface functional groups resulted in the aggregation of polysaccharide chains forming into a smaller steric hindrance (Zhan et al., 2022). This feature makes polysaccharides enter the cell smoothly to bind to receptors on the surface of macrophages, thus activating the release of related factors.

4.3. Effect of the acetylation of polysaccharides on immunomodulation

Acetylated *Cyclocarya paliurus* polysaccharide with a substitution degree of 0.13 could activate peritoneal macrophages and stimulate the secretion of IL-1β, IL-6, and TNF-α (Liu et al., 2017). Acetylated polysaccharide from *Chlorella pyreoidosa,* with acetyl groups attached to the O-2 and O-4 positions on Gal*p* residues was proved to possess immunologically active (Yuan et al., 2020). Acetylated *Morchella angusticeps* peck polysaccharide showed a stronger ability to suppress the overproduction of NO and TNF-α by down-regulating the level of inducible nitric oxide synthase (iNOS), and p38 via nuclear factor κB (NF-κB) and p38/mitogen-activated protein kinase signaling pathways. It was found that a substitution degree in the range of 0.2–0.4 was the most appropriate for its immune activation and anti-inflammatory activity (Yang et al., 2019b). Acetylated β-(1 → 4)-D-mannans showed stronger immunostimulatory activity than non-acetylated β-(1 → 4)-D-mannans. Acetylation groups may explain this, which is an essential feature related to immunostimulatory function (Leung et al., 2004). Thus, after acetylation, the polysaccharide has both the C═O properties of esters and the C–O properties of carbonyl groups to increase the solubility of fats. According to the principle of "like dissolves like", it may better enter the cell membrane which mainly consists of phospholipid, and then get into nucleus to regulate the transcription of related genes (Ren et al., 2007). Based on the above studies on the optimal acetyl substitution degree of polysaccharides, the appropriate DS for acetylation for the high activity is 0.1–1.

Above all, chemical modification of polysaccharides can change their structure to enhance the immunostimulatory bioactivities by adding the substitute group. Sulfation, selenylation and acetylation have different mechanisms to activate immune cells. These modifications change the physicochemical properties and biological activities of polysaccharides. It is essential to learn more about polysaccharide modification since natural polysaccharides often do not meet the applicable standards.

5. Effect of glycosidic bond types of polysaccharides on immune activity

5.1. Immune-enhancing activity of β-1,3-D-glucosyl

β-1,3-D-glucan from *Saccharomyces cerevisiae* affects the immunity of S180 tumor-bearing mice and tumor-bearing hosts. The results showed that β-1,3-D-glucan can significantly enhance the immune response (Mo et al., 2017). β-(1 → 3,6)-glucan extracted from *Russula vinosa* Lindblad has good immunostimulatory activities, and it has one β-1,3-glucan main chain and two β-glucosyl side-branching units (Zhang et al., 2022). β-1,3-D-glucan extracted from lentinan exhibits strong anti-cancer activity by stimulating the human immune system. Its specific binding mode with the Dectin-1 receptor on immune cells is shown in Fig. 1C (Zhang et al., 2011). *Durvillaea antarctica* polysaccharide is β-1,3-glucan with β-1,6-branches, which can enhance proinflammatory immune cells activity and has about a 50% tumor growth inhibition rate (Su et al., 2019). *A. auricula-judae* polysaccharide is β-1,3-D-glucan with two β-1,6-D-glucosyl side chains for every three main chains glucose residues, which enhances the immune-response by inducing apoptosis in tumor cells and inhibiting angiogenesis in tumor tissues (Ping et al., 2016). The main chains containing β-1,3-glucosyl may greatly stimulate the number of various types of human immune cells and enhance activities in response to cancer cells (Wasser, 2002). Furthermore, the side chain substituted at the O-6 position of the β-1,3-glucosyl backbone will increase the water solubility of β-1,3-D-glucan, which would be crucial for its biological properties.

5.2. Immune-enhancing activity of α-1,4-D-glucosyl

The medicinal plant *Tinospora cordifolia* polysaccharide exhibited unique immunostimulatory properties. It has a structural form of α-1,4-glucan that activated different subsets of the lymphocytes, such as natural killer (NK) cells, T cells, and B cells (Nair et al., 2004). Radix ginseng Rubra polysaccharide with main chain α-1,4-glucosyl and 1,4,6-α-glucosyl as branching units, activated macrophages. The changes in the NO levels and the expressions of IL-6, IL-12, and TNF-α were found to be upregulated after treatment of Radix ginseng Rubra polysaccharide (Zhang et al., 2021c). The main chain of neutral polysaccharides derived from ginger is α-1,4-D-glucosyl, which showed significant immune activity, promoting the proliferation of macrophages and the secretion of immune substances (Yang et al., 2021). The α-1,4-glucan from *Pseudallescheria boydii* displayed a remarkable immunological activity via activating toll-like receptors and fungal phagocytosis, and its binding pattern to TLR2 is shown in Fig. 1D (Bittencourt et al., 2006). The α-1,4-glucan, derived from *Actinidia chinensis* root, is a potential immune-promoting agent, exerting stimulatory effects on phagocytosis activity and NO production by macrophages (Niu et al., 2016).

5.3. Immune-enhancing activity of β-1,4-D-glucosyl

Polysaccharides that possess β-1,4-D-glucosyl-branched-β-1,6-D-glucosyl residues have effects in performing immunological functions in the research of *Rhizobium* sp.N613 exopolysaccharide. It was observed that tumor formation decreased significantly and serum hemolysis antibody increased dramatically after being treated with *Rhizobium* sp. N613 exopolysaccharide (Zhao et al., 2010). The same conclusion was achieved in the study of polysaccharides from *Colocasia esculenta* (taro), which enhanced the phagocytosis ability of macrophages and promoted NO, TNF-α, and IL-6 production by recognition of TLR-4 and TLR-2 (Li et al., 2018a). β-1,4-glucan from *Antrodia cinnamea* has two long β-1,6 branches in each repeating unit, and has achieved better efficacy in performing immune activity against tumor growth (Lin et al., 2019).

5.4. Immune-enhancing activity of α-1,6-D-glucosyl

α-1,6-D-glucan isolated from bananas could elevate T cell proliferation, phagocytic function of macrophages, and hemolysin antibody levels, and might be the active substance responsible for the health benefits of bananas (Yang et al., 2019a). Longan polysaccharide was also detected as α-1,6-D-glucan due to its unique linkage →6)-D-glucosyl-(1 → . This should be the specific bioactive linkage that plays a leading role in the health function of longan (Zhu et al., 2013). Polysaccharide from *Cistanche deserticola* was found to display greater potential for incrementation of the splenocyte as α-1,6-D-glucan (Wu and Tu, 2005). All these above results indicated that α-1,6-D-glucan was a good immunomodulator.

5.5. Immune-enhancing activity of β-1,4-D-mannosyl

The backbone of the modified Aloe polysaccharides mainly consists of β-1,4-D-mannosyl, exhibiting potent macrophage-activating activity by increasing cytokine production, nitric oxide release, expression of surface molecules, and phagocytic activity (Im et al., 2005). The galactomannans purified from coffee infusions composed of β-1, 4-D-mannose branched with α-1,6-D-galactose and α-1,6-D-arabinose, presenting *in vitro* immunostimulatory activity on murine B lymphocytes and T lymphocytes (Simões et al., 2010). β-1,4-D-acemannans isolated from *Dendrobium devonianum* stem possess immunomodulatory activities against the growth of HepG2 and MCF-7 cancer cells. It has a backbone chain composed of →4)-β-D-Man*p*-(1→ residue with internal →4)-2-O-acetyl-β-D-Man*p*-(1→, →4)-3-O-acetyl-β-D-Man*p*-(1→, and non-reducing end β-D-Man*p*-(1→ residues (He et al., 2022).

5.6. Immune-enhancing activity of β-1,4-D-xylopyranosyl

Arabinoxylans from wheat bran consist of backbone chains of β-(1–4)-D-xylopyranosyl residues to which α-L-arabinofuranose units are linked as side chains. They have powerful stimulant effects on innate and acquired immune responses by macrophage phagocytosis and delayed hypersensitivity reaction (Zhou et al., 2010). Corn husk arabinoxylan, with a backbone of β-(1,4)-linked xylose residuesmainly through α-(1,3) arabinofuranose residue, significantly enhanced the production of IL-2 and with a slight increase in IL-4 in mitogen-induced proliferation of spleen cells (Ogawa et al., 2005). *Cassia obtusifolia* seeds polysaccharides were investigated to possess immunomodulatory activity by promoting phagocytosis and stimulating the production of NO and cytokines (TNF-α and IL-6) (Feng et al., 2018). These structures were elucidated to be glucuronoxylan, with glucopyranosyluronic acid group terminally attached to O-2 of the →4)-β-Xylp-(1→ (Feng et al., 2016).

Polysaccharides containing β-1,3-D-glucosyl form a triple-helix conformation, that promotes TNF-α release by monocyte or macrophage cells (Ferreira et al., 2015). β-1,4-glucans can be recognized in TLR-2 and TLR-4 which contribute to satisfactory immunological activity. The α-1,4-glucosyl may account for their activation of macrophages by increasing cellular pseudopods visibly and changing into irregular shapes. Linear α-1,6-glucans without branching activate lymphocytes and macrophages to resist foreign pathogens and irritants by secreting cytokines indirectly. Mannoses located at the terminus of β-1, 4-D-mannose may bind to the mannose receptor, and these protein-polysaccharide interactions are important in innate immune responses and pathogen recognition and uptake (Cummings, 2022). Xylans consisting of backbone chains of β-(1–4)-D-xylopyranosyl residues have been shown to possess the ability to induce a range of immune responses. In human macrophages, their binds with macrophages are mainly dependent on Dectin-1b (Moerings et al., 2022). Due to their complex glucosidic linkage structures, immunostimulatory mechanism research and application of polysaccharides may face great challenges. Therefore, providing a detailed analysis of polysaccharide structure is essential for an in-depth study of their structure-activity relationships. The detailed glucosidic linkage of above polysaccharides is shown in Table 2, and the illustrations of them are shown in Fig. 3.

6. Effect of chain conformation of polysaccharides on immune activity

6.1. Triple-helix structure of polysaccharides with immune-enhancing effects

The intermolecular hydrogen bonds and molecular interactions between polysaccharides and solvent molecules bind the polysaccharide three chains together (Okobira et al., 2008). Both HEB-NP Fr I and HEB-AP Fr I are polysaccharides extracted from *Hericium erinaceus*, but HEB-AP Fr I has a three-dimensional structure of β-1,3-branched-β-1, 2-mannan, and has a better promotion of macrophages activity (Lee et al., 2009). Lotus leaf polysaccharides LLWP-1 and LLWP-3 can both promote the proliferation of macrophages, yet LLWP-3 has stronger immune potential, which may be due to LLWP-3 having a triple helical structure while LLWP-1 do not (Song et al., 2019b). β-D-glucans with a triple helix structure can better promote TNF-α release (Falch et al., 2000; Satitmanwiwat et al., 2012). Generally D-glucans with high branching degrees are contributed to the formation of triple helices, which is positively associated to immunostimulatory activity (Zhang et al., 2015; Zhao et al., 2014). The stem lettuce polysaccharides, with triple-helical chains, exert strong immune-enhancing by promoting the proliferation of macrophages without cytotoxicity (Nie et al., 2018). Lentinan polysaccharides with different chain conformations had different immune activities, and triple-helical lentinan polysaccharide possesses better immunity activation than the same polysaccharide with single random coils (Zhang et al., 2011). A novel acid polysaccharide with a triple-helix conformation was isolated from the pulp of *Rosa laevigata* Michx fruit, which could activate the mitogen-activated protein kinase (MAPK) and NF-κB signaling pathways. Its immunomodulatory activity was found to be dependent on the helical conformation and the presence of hydrophilic groups on the outer surface of the helix (Zhan et al., 2020). α-glucan from fruiting bodies of *Volvariella volvacea* improves the phosphorylated levels of p38, JNK and ERK in macrophage cells to promote the secretion of NO, TNF-α, IL-6 and IL-1β (Cui et al., 2020). The higher ratio of side glucose residues to the backbone glucose residues in the repeating unit, the smaller pitch of the helix structure, and the larger the hydrophobic cavity formed by the triple-helix chain, which is greatly related to molecular stiffness (Okobira et al., 2008). Furthermore, the triple-helix polysaccharide is better recognized by the receptors of immune cells for its higher stiffness.

6.2. Random coil structure of polysaccharides with immune-enhancing effects

Cordyceps militaris polysaccharide with random coil conformation of β-1,4-branched-β-1,6-galactoglucomannan has high immunostimulatory

Table 2
Glucosidic linkage of polysaccharides with immune-enhancing effects.

Types of glucosidic linkage	Types of polysaccharides	Source	Concrete glucosidic linkage	References
β-1,3-D-glucosyl	β-1,3-D-glucan	*Saccharomyces cerevisiae*	[3)-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1→]n	Mo et al. (2017)
	β-(1 → 3,6)-glucan	*Russula vinosa* Lindblad	a β-1,3-glucan backbone with two β-glucosyl side-branching units at O-6 position by every five backbone residues	Zhang et al. (2022)
	Lentinan polysaccharides		β-1,3-D-glucan having (1 → 6)-glucosyl side groups	Zhang et al. (2011)
	β-1,3-glucan	*Durvillaea antarctica*	β-1,3-glucan with β-1,6-branches	Su et al. (2019)
	β-1,3-glucan	*A. auricula-judae*	β-1,3-D-glucan with two β-1,6-D-glucosyl side chains for every three main chain glucose residues	Ping et al. (2016)
α-1,4-D-glucosyl	α-D-glucan	Medicinal plant *Tinospora cordifolia*	(1 → 4) linked backbone and (1 → 6) linked branches	Nair et al. (2004)
	Radix ginseng Rubra polysaccharide		α-1,4-glucan, with a 1,4,6-α-glucosyl branch unit	Zhang et al. (2021c)
	Neutral ginger polysaccharide		α-1,4-glucan and α-D-glucosyl residues branched at C-6 position	Yang et al. (2021)
		Pseudallescheria boydii	linear α-1,4-D-glucosyl residues substituted at C-6 position with α-D-glucosyl branches	Bittencourt et al. (2006)
		Actinidia chinensis root	α-D-glucan consisting of predominant 4-linked α-D-glucosyl residues branched at O-6.	Niu et al. (2016)
β-1,4-D-glucosyl	Rhizobium exopolysaccharide	*Rhizobium* sp.N613	a backbone of β-D-1,4-glucosyl residues and branches of β-D-1,6-glucosyl residues	Zhao et al. (2010)
	β-1,4-D-glucan	Taro	β-1,4-D-glucosyl-branched-β-1,6-D-glucosyl residues	Li et al. (2018a)
	β-1,4-glucan	*Antrodia cinnamea*	β-1,4-glucan with two long β-1,6 branches in each repeating unit	Lin et al. (2019)
α-1,6-D-glucosyl	α-1,6-D-glucan	Banana	→6)-α-D-glucosyl-(1→	Yang et al. (2019a)
	α-1,6-D-glucan	Longan	→6)-D-glucosyl-(1→	Zhu et al. (2013)
	α-1,6-D-glucan	*Cistanche deserticola*	→6)-D-glucosyl-(1→	Wu and Tu. (2005)
β-1,4-D-mannosyl	Aloe polysaccharides	Aloe	β-1,4-D-Man*p* backbone	Im et al. (2005)
	Galactomannans	Coffee	β-1,4-D-Man*p* branched with α-1,6-D-galactose and α-1,6-D-arabinose	Simões et al. (2010)
	Acemannan	*Dendrobium devonianum* stem	A backbone of →4)-β-D-Manp-(1 → residue with internal →4)-2-O-acetyl-β-D-Manp-(1→, →4)-3-O-acetyl-β-D-Manp-(1→, and non-reducing end β-D-Manp-(1 → residues	He et al. (2022)
β-1,4-D-Xylopyranosyl	Arabinoxylans	Wheat bran	β-(1–4)-linked D-xylopyranosyl backbone chains and α-L-arabinofuranose side chains	Zhou et al. (2010)
	Arabinoxylan	Corn husk	A backbone of β-1,4-D-Xylopyranosyl and branches of α-(1,3) arabinofuranose residues	Ogawa et al. (2005)
	Glucuronoxylan	*Cassia obtusifolia* seeds	β-1,4-D-Xylopyranosyl backbone and glucopyranosyluronic acid group branches	Feng et al. (2016)

activity by activating macrophages (Lee et al., 2010). Studies showed that the chain conformation of pectin polysaccharide with immune activation is irregular conformation (Suárez et al., 2006; Yin et al., 2012). A purified polysaccharide (BDP) from the injection powder of *Bacillus* Calmette Guerin and nucleic acid has revealed that it can exert immune function by stimulating the production of NO and activating the function of macrophages by promoting cytokine. Structural analyses showed that BDP is branched and flexible random coils (Liu et al., 2016). *Paecilomyces cicadae* heteropolysaccharide presents notable effects on activating macrophages through the TLR-4 signaling pathway. It exists as a flexible chain conformation, and molecular docking with TLR-4 is shown in Fig. 1B (Wei et al., 2016). It is noteworthy that immune-enhancement has been reported to correlate with the structure of random coil, which has less steric hindrance and a large internal degree of rotational freedom. These two properties reduce the spatial obstruction of polysaccharides when approaching immune cells, and allow a better activation of their immune activity.

Taken together, the chain conformation is also critical for polysaccharides exhibiting their biological activity. Polysaccharide conformation is related to the spatial arrangement of the atoms that may result in its possible immune-stimulating activity. Different conformations may result in different activities due to their linkage patterns, branched structures, branching degrees, intermolecular hydrogen, and electrostatic repulsion of substituents. In summary, the triple-helix structure exhibits much stronger bioactivity than the random coil structure, as its helical structure forms a rigid tertiary conformation. And the interactions between the tertiary conformation result in the formation of a network/quaternary structure, which scattered polysaccharide particles rotate and shrink into clusters to stronger shear thinning (Wang et al., 2021). Shear-thinning refers to the fact that the dispersed polysaccharides particles rotate and contract into clusters, reducing their mutual hooks and facilitating contact with immune cells.

7. Effect of branching degrees of polysaccharides on immune activity

Polysaccharides have some branches connected to the main chain or have a linear backbone without branches. The branching degree of polysaccharides is related to the monosaccharide residues and branches connected to the main chain. *P. oxalicum* intracellular polysaccharide (IPS) has a stronger inhibitory effect on galactose lectin than *P. oxalicum* extracellular polysaccharide (EPS), studies showed that it was due to the difference of branching degree of the polysaccharides, where the branching degree of IPS is 17.2% and EPS is 7.9% (Zhang et al., 2021b). Both GPNE-I and GPNE-II are polysaccharide components extracted from ginseng neutral polysaccharides, with the branching degree of 38.17% and 50.78% respectively. Different branching degrees, might lead to variations in their activities by stimulating lymphocyte proliferation (Li et al., 2019). The main chains of both CVPn and CVPa from *Coriolus versicolor* dried fruiting bodies polysaccharides are (1 → 3)-β-D-glucopyranosyl group, but CVPa has less branching degrees, exhibiting more obvious induction of NO production, augmentation of iNOS and phagocytosis of macrophage cells (Zhang et al., 2021c). Higher branching degrees in *Polygonum multiflorum* polysaccharides

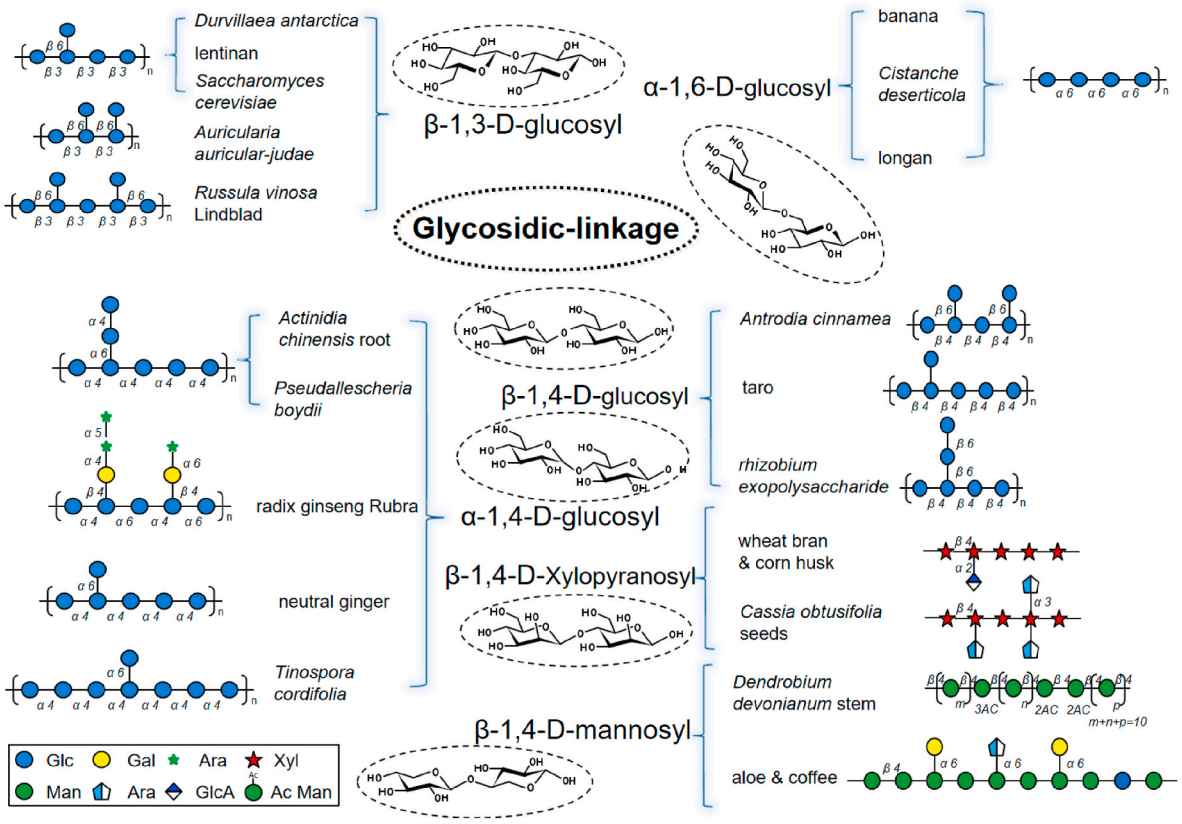

Fig. 3. The illustration of glucosidic-linkage in polysaccharides with immune-enhancing effects.

were suggested to be the positive characteristics for a stronger activation effect on phagocytosis of peritoneal macrophages (Zhang et al., 2018). *Prunella vulgaris* Linn polysaccharides with higher degree of branching showed the stronger immunomodulatory activities by releasing NO, TNF-α, and IL-6 (Li et al., 2015). Above all, branching degree is an important factor that affects the biological functions of polysaccharides, and this correlation is not linear. The reason for this phenomenon is that the moderate branching degree may enhance the affinity to the receptor in immune cells (Mueller et al., 2000).

In summary, polysaccharides have a wide range of sources and diverse structures, and their structure-activity relationships of immunity are complex. Therefore, the single and the combinations of these structural features seem to influence their immune activity. The molecular weight, monosaccharide domain, chemical modification, glycosidic bond composition, chain conformation and branching degrees will all play an impact on immunological activity (Box 1).

8. Conclusions

The appropriate molecular weight of polysaccharides between 10 and 1000 kDa can be easily diffused and absorbed into immune cells. When monosaccharide components such as galactose, mannose, rhamnogalacturonan-I, arabinogalactan, and uronic acid with a specific ratio contained, the polysaccharides would have immune-regulatory activity because these structural features may trigger signaling pathways on the surface of immune cells ane then have an impact on downstream immune responses. The chemical modification changes the physicochemical properties and biological activities of original polysaccharide to meet application requirements, especially ideal DS may maximize the immunomodulatory effect. Polysaccharides with β-1,3-D-glucosyl form into triple-helix while β-1,4-glucans can be recognized in TLR-2 and TLR-4. The α-1,4-D-glucosyl linkage changes the shape of macrophages to activate them and linear α-1,6-D-glucans may activate immune cells due to their rare structues without branching. β-1,4-D-mannoses expressing terminal mannose may be bind with mannose receptor on the surface of immune cells while xylans consisting of backbone chains of β-(1–4)-linked D-xylopyranosyl residues have shown to mainly bind with Dectin-1b. Triple-helix polysaccharide is better recognized by the receptors of immune cells due to its higher stiffness, while random coil polysaccharide possesses smaller steric hindrance making it easier to enter immune cells. Polysaccharides with a moderate branching degree possess suitable space size, enhancing the affinity to the receptor in macrophages to release immune-associated factors. The relationship between the chemical structure of polysaccharides and their immune activity were discussed, including how to bind the immune cells via the molecular docking. And the polysaccharide structural features caused the immune response are summarized in Box 1. Moreover, the research results can also guide the production direction of immunological drugs related to medicine and provide theoretical support for the development of novel food additives.

CRediT authorship contribution statement

Ruoxin Chen: Writing – original draft, Formal analysis, Data curation. **Jingxiang Xu:** Formal analysis, Writing – review & editing. **Weihao Wu:** Formal analysis, Writing – review & editing. **Yuxi Wen:** Writing – review & editing. **Suyue Lu:** Writing – review & editing. **Hesham R. El-Seedi:** Writing – review & editing. **Chao Zhao:** Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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