Mechanism of Antioxidant Action of Plant Polysaccharides via NF- B and MAPK/Nrf2 Signaling Pathways: A Postprint

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Abstract

Plant polysaccharides are one of the important active components in plant extracts, possessing multiple biological activities such as antioxidant, immune-enhancing, and antitumor effects. Regarding antioxidant activity, plant polysaccharides reduce the secretion of inflammatory factors through the nuclear factor B (NF- B) signaling pathway, thereby alleviating oxidative stress damage; they also scavenge free radicals in the body or cells via the mitogen-activated protein kinase/nuclear factor erythroid 2-related factor 2 (MAPK/Nrf2) signaling pathway, increasing the activity or content of antioxidant enzymes and enhancing the resistance of the body or cells to oxidative stress. This article primarily reviews the causes and hazards of oxidative stress, and elaborates on the current research status and existing problems concerning the roles of NF- B and MAPK/Nrf2 signaling pathways in the antioxidant activity of plant polysaccharides, providing theoretical references for the research and application of plant polysaccharides as antioxidants.

Full Text

Mechanism of Antioxidant Action of Plant Polysaccharides via Nuclear Factor B and Mitogen-Activated Protein Kinase/Nuclear Factor Erythroid 2-Related Factor 2 Signaling Pathways

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Abstract

Plant polysaccharides, one of the principal bioactive components in plant extracts, exhibit multiple biological functions including antioxidant, immunomodulatory, and antitumor activities. Regarding antioxidant effects, plant polysaccharides mitigate oxidative stress damage by reducing inflammatory factor secretion through the nuclear factor B (NF- B) signaling pathway, and scavenge free radicals in the body or cells while enhancing antioxidant enzyme activity and content via the mitogen-activated protein kinase (MAPK)/nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, thereby strengthening resistance to oxidative stress. This review summarizes the causes and detrimental effects of oxidative stress, elaborates on the current research status and existing challenges concerning the roles of NF- B and MAPK/Nrf2 signaling pathways in mediating the antioxidant actions of plant polysaccharides, and provides a theoretical reference for future research and application of plant polysaccharides as antioxidant agents.

Keywords: plant polysaccharide; mitogen-activated protein kinase; nuclear factor erythroid 2-related factor 2; nuclear factor B; antioxidant action

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During livestock growth and development, environmental changes, dietary alterations, diseases, and xenobiotic metabolism can induce oxidative stress, characterized primarily by the generation of excessive free radicals dominated by reactive oxygen species (ROS). Under normal conditions, animals possess an antioxidant defense system capable of counteracting oxidative stress damage, and low-intensity oxidative stress can activate cellular or systemic antioxidant defenses by increasing antioxidant enzyme activity or reducing oxidase activity. However, when stress intensity persists and escalates, the balance between oxidants and antioxidants is disrupted, overwhelming the body' s regulatory capacity.

Supplementation with exogenous bioactive substances or antioxidants represents an effective strategy to alleviate oxidative stress. In production practice, chemically synthesized antioxidants are commonly used, but these suffer from drawbacks such as heat instability, carcinogenic potential, and drug residues. Consequently, identifying cost-effective, efficient, and pollution-free plant-derived antioxidants has become a practical and effective approach to mitigating oxidative stress in livestock production.

1. Special Structure of Plant Polysaccharides

Plant polysaccharides are important bioactive components in plant extracts with diverse biological activities that are closely associated with their unique primary and advanced structures. The primary structure encompasses monosaccharide composition, glycosyl arrangement, glycosidic bond types, chain length, and functional groups. Plant polysaccharides are composed of monosaccharides such as glucose, fructose, galactose, arabinose, rhamnose, and xylose. Unlike conventional carbohydrates such as starch, plant polysaccharides are typically linked via -1,4-, -1,3-, -1,4-, and -1,6-glycosidic bonds. Structural units may form linear chains or branched structures, with linear chains generally connected by -1,4- and -1,4-glycosidic bonds, while branch points commonly involve -1,6-glycosidic bonds. The primary structure is further complicated by functional groups attached to sugar moieties, including phosphate, sulfate, and methylated groups. Advanced structures arise from non-covalent interactions among side chains based on the primary structure. At the secondary level, polysaccharide backbone chains aggregate through hydrogen bonding. Tertiary structure represents the spatial conformation formed by further coiling and folding of the secondary structure, while quaternary structure refers to aggregates formed by non-covalent association of multiple polysaccharide chains. The degree of branching is intimately correlated with biological activity, and different branching patterns produce distinct bioactivities, making it a critical determinant for achieving specific biological effects. Additionally, molecular weight, solubility, viscosity, degree of polymerization, and metal ion chelation capacity also influence polysaccharide bioactivity to varying extents.

2. Causes and Hazards of Oxidative Stress

Oxidative stress arises from an imbalance between oxidants and antioxidants in cells or organisms. In livestock production, environmental temperature fluctuations, dietary changes, suboptimal management practices, and diseases can trigger free radical accumulation and oxidative stress. The inherent antioxidant defense system can counteract free radicals by increasing antioxidant enzyme secretion or activity, but when free radical production exceeds defensive capacity, oxidative damage occurs. Such damage primarily manifests in three aspects: DNA modification, protein carbonylation, and lipid peroxidation leading to malondialdehyde (MDA) formation.

Under inadequate management conditions, high stocking density, poor ventilation, lack of exercise, and high-energy diets make oxidative stress ubiquitous in animal production, compromising immunity and increasing susceptibility to enteritis, pneumonia, postpartum sepsis, mastitis, and other diseases. Exogenous antioxidants including vitamins (C, E), trace elements (selenium, zinc), synthetic antioxidants [ethoxyquin (EMQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA)], and plant-derived antioxidants (proanthocyanidins, resveratrol) are used to prevent feed oxidation and enhance antioxidant capacity. However, due to unpredictable stress levels and relatively high costs of some antioxidants, synthetic antioxidants are often applied excessively in practice, leading to residues in animal products and environmental pollution. Therefore, developing affordable, green, and non-polluting antioxidant alternatives is critically important.

3. Manifestations of Antioxidant Action of Plant Polysaccharides

Research on the antioxidant properties of plant polysaccharides has become increasingly sophisticated, with effects manifested primarily in two aspects. First, direct or indirect free radical scavenging: transition metals containing unpaired electrons participate in numerous free radical reactions, such as acting as electron carriers in phosphorylation processes. Free transition metals, particularly iron ions, can undergo chelation reactions with ROS to generate additional free radicals. Plant polysaccharides contain alcoholic hydroxyl groups that can chelate metal ions such as ferrous (Fe^{2}) and copper (Cu^{2}) ions required for ROS generation, thereby scavenging free radicals. For instance, polysaccharides from *Sansevieria trifasciata* and perilla leaves can scavenge hydroxyl radicals (·OH), superoxide anions (O ·), 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH·), nitrite ions, and chelate iron ions. Second, enhancing antioxidant enzyme activity or reducing oxidase activity: nuclear factor erythroid 2–related factor 2 (Nrf2) is a crucial transcription factor that binds to antioxidant response elements in the nucleus to initiate antioxidant enzyme gene expression. Plant polysaccharides can modulate the Nrf2/Kelch-like ECH-associated protein 1 (Keap1) signaling pathway to upregulate antioxidant enzyme gene expression. Since inflammatory responses elevate intracellular free radical levels and induce oxidative stress, studies have shown that astragalus polysaccharides can increase superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities while decreasing MDA content, and alleviate oxidative stress damage by inhibiting NF- B signaling to reduce interleukin-8 (IL-8) gene expression.

4. Mechanisms and Main Signaling Pathways of Antioxidant Action

The mechanisms by which plant polysaccharides regulate antioxidant action primarily involve two aspects: (1) modulation of inflammatory response through the NF- B signaling pathway to regulate inflammatory factor gene expression and mitigate oxidative stress damage; and (2) activation of the MAPK/Nrf2 signaling pathway to promote phase II detoxifying enzyme secretion, enhance antioxidant enzyme production and activity, reduce free radical levels, and strengthen oxidative stress resistance [Figure 1: see original paper].

Figure 1 Mechanisms of resisting oxidative stress by plant polysaccharides [9-11]

4.1 NF- B Signaling Pathway

Toll-like receptors (TLRs) are a family of innate immune recognition receptors that induce cytokine secretion, including intracellular compartment-localized TLR3, TLR7, TLR8, and TLR9 (in endoplasmic reticulum, etc.) and cell membrane-localized TLR1, TLR2, TLR4, TLR5, and TLR6. Upon TLR4 activation, myeloid differentiation factor 88 (MyD88), interleukin-1 receptor-associated kinase 1 (IRAK1), and tumor necrosis factor receptor-associated factor 6 (TRAF6) are recruited for intracellular signal transduction. TLR4 regulates both MAPK and NF- B signaling components, promoting NF- B translocation into the nucleus to control downstream gene expression.

The NF- B pathway is a primary inflammatory signaling cascade normally inhibited by I B proteins. Under oxidative stress, I B undergoes phosphorylation and ubiquitination, leading to its degradation and releasing NF- B for nuclear translocation to regulate inflammatory gene expression. Plant polysaccharides bind to and activate TLRs on the cell surface, modulating downstream gene expression. Exacerbated inflammatory responses cause free radical accumulation at injury sites, while inflammatory cytokines such as interleukin-6 (IL-6) and chemokine IL-8 not only regulate inflammation but also initiate and maintain cellular senescence through NF- B-dependent gene expression. Retinoic acid-inducible gene 1 (RIG-1) is associated with inflammation and represents an important signaling pathway in senescence-related inflammatory responses, with upregulated RIG-1 expression observed in various chronic inflammatory conditions. IL-6 and IL-8 expression is primarily controlled by NF- B and activator protein 1 (AP-1), and in senescent cells, RIG-1 modulates NF- B signaling to influence IL-6 and IL-8 expression.

Plant polysaccharides can alleviate oxidative stress damage by regulating inflammatory responses. Astragalus polysaccharides significantly reduce serum inflammatory cytokine levels, demonstrating anti-inflammatory effects that mitigate oxidative stress-induced inflammatory damage in myocardial tissue of severely burned rats. These polysaccharides also inhibit isoproterenol-induced cardiac hypertrophy in rats by suppressing the TLR4/NF- B pathway. Research shows that *Salvia miltiorrhiza* polysaccharides activate NF- B to promote tumor necrosis factor- (TNF-) secretion and enhance macrophage immune function, while dandelion polysaccharides inhibit NF- B phosphorylation to alleviate oxidative damage. Collectively, these findings demonstrate that plant polysaccharides can mitigate oxidative damage by suppressing inflammatory factor expression under oxidative stress conditions, while upregulating inflammatory gene expression to enhance immune function under normal physiological states.

4.2 MAPK/Nrf2 Signaling Pathway

The MAPK pathway responds to various stimuli including oxidative stress and comprises three major branches: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38. ERK primarily regulates growth hormone and factor gene expression, whereas JNK and p38 are critical components in oxidative stress responses. Under normal conditions, Nrf2 is coupled with Keap1 in the cytoplasm and maintained in an inhibited state. Upon activation by plant polysaccharides, Nrf2 dissociates from Keap1, translocates into the nucleus, and binds to antioxidant response elements (ARE) to encode phase II detoxifying enzyme genes, thereby enhancing oxidative stress resistance. Plant polysaccharides can induce Nrf2 pathway activation through the MAPK signaling cascade to regulate phase II detoxifying enzyme expression.

The MAPK/Nrf2 signaling pathway is crucial for oxidative stress responses and has been extensively studied in plant polysaccharide research. Under oxidative stress, this pathway is activated to promote expression of phase II detoxifying enzyme heme oxygenase-1 (HO-1). Dandelion polysaccharides can activate Nrf2 via the MAPK pathway to induce HO-1 expression, reduce intracellular ROS production, and promote synthesis of antioxidant enzymes such as SOD and GPX, thereby enhancing oxidative stress resistance. Plant polysaccharides maintain the redox balance in cells and organisms through MAPK/Nrf2 signaling to exert antioxidant effects.

5. Current Application Status

Due to their antimicrobial, antioxidant, and immunomodulatory properties, plant polysaccharides have become a research focus for their ability to inhibit oxidases and activate antioxidant enzymes. However, their mechanisms of action and application standards remain unclear, with related research still in the exploratory stage.

TLR4 is the primary cell membrane receptor activated by plant polysaccharides. Recent studies have identified that plant polysaccharides with specific structures (TLR4-related immunopolysaccharides) can activate TLR4 synthesis on the cell surface and trigger immune and antioxidant signaling pathways. These polysaccharides are composed of galactose, rhamnose, glucose, and arabinan. Polysaccharides from safflower, apple, *Angelica sinensis*, astragalus, and *Lycium barbarum* have all been shown to be recognized by TLR4, enhance immunity, and alleviate oxidative damage.

Currently, the most studied plant polysaccharides include astragalus polysaccharide, alfalfa polysaccharide, *Acanthopanax senticosus* polysaccharide, and *Lycium barbarum* polysaccharide. Astragalus, a common traditional Chinese medicine for tonifying qi and blood, enhances immunity and reduces fatigue, with astragalus polysaccharide being one of its main active components. Studies demonstrate that astragalus polysaccharide can inhibit mitochondrial dysfunction induced by free radicals and oxidative damage. Alfalfa, known as the "king of forages" due to its excellent quality, high protein content, strong adaptability, and high yield, contains alfalfa polysaccharide as a key active component. Research shows that alfalfa polysaccharide can scavenge DPPH · radicals, improve growth performance and antioxidant capacity in heat-stressed rabbits, and reduce oxidative stress-induced liver cell damage in broilers. *Acanthopanax senticosus*, a classic herbal medicine recorded in the *Compendium of Materia Medica* for treating rheumatism and hypertension, contains polysaccharides that can scavenge DPPH · radicals and enhance antioxidant capacity. *Lycium barbarum* polysaccharide exhibits anti-aging effects by promoting Nrf2 nuclear translocation, upregulating HO-1 gene expression, and strengthening oxidative stress resistance.

6. Summary and Outlook

Plant polysaccharides are major active components in plant extracts with important practical significance for antibiotic-free livestock production as currently required. China has a long history of herbal medicine application and abundant plant resources, including traditional Chinese medicinal herbs. The studied polysaccharides such as astragalus and *Lycium barbarum* polysaccharides were discovered and applied from these herbs, offering broad prospects for research on plant polysaccharide application in livestock production, particularly under the trend of grain-to-forage conversion.

However, research on plant polysaccharides remains in its infancy, with several unresolved issues in practical application. First, extraction technologies are immature and lack a complete, rigorous standardized system. Second, the extensive use of organic solvents in extraction processes significantly affects the palatability of polysaccharide feed additives, and whether this influences feed intake requires further investigation. Third, the inconsistent concentration of extracted polysaccharides necessitates determination of appropriate dosage levels for livestock production. Finally, the potential carcinogenic hazards of residual organic solvents in plant polysaccharides to animals remain unclear, making solvent removal an urgent problem to solve for widespread application.

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