Plant Science 208 (2013) 42–49

Contents lists available at SciVerse ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review

Multiple effects of chitosan on plant systems: Solid science or hype☆

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ARTICLE INFO

Article history:
Received 15 November 2012
Received in revised form 1 March 2013
Accepted 6 March 2013
Available online 21 March 2013

Keywords:
Chitin
Pathogenesis-related (PR) genes
Chitosan oligomers
Innate immunity
Non-host resistance
Plant defense

ABSTRACT

Chitosan, a naturally occurring polymer, became available in the 1980s in industrial quantities enabling it to be tested as an agricultural chemical. A usual procedure for developing agricultural chemicals starts by testing a number of different chemically synthesized molecules on a targeted biological system. Alternately, chitosan has been investigated as a single natural molecule assayed with numerous biological systems. This report describes the unique properties of the molecule and its oligomers, primarily in plant defense, additionally in yield increase, induction of cell death and stomatal closing. The plant plasma membrane and nuclear chromatin have been proposed as targets, though chitosan oligomers enter most regions of the cell. Subsequent changes occur in: cell membranes, chromatin, DNA, calcium, MAP kinase, oxidative burst, reactive oxygen species (ROS), callose, pathogenesis related (PR) genes/proteins, and phytoalexins. Chitosan oligomer mode(s) of action are proposed for different plant systems. Chitosan efficacy was based on documentation from published data. Attention was given to how chitosan, either applied externally or released by fungal inoculum, is transferred into plant cells and its subsequent action upon membrane and/or chromatin components. Within is a proposed scheme describing chitosan generation, signaling routes and mechanisms of defense gene activation. Examples of beneficial chitosan applications to major crop/food plants were included.

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☆ “hype” = Excessive publicity or exaggerated claims.

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http://dx.doi.org/10.1016/j.plantsci.2013.03.007

1. Introduction

Chitosan has been available as a commercial product for 3 decades. Its parent compound, chitin, is present in the waste exoskeleton material of crab and shrimp harvests. The prospects for bringing value to these wastes motivated world wide research to find applications for chitin and its de-acetylated derivative, chitosan. As a result applications ranging from cosmetics, medicine, materials and agriculture have been forth coming and reported world wide [1–3].

With the diversity of applications, research reports and financial interests oriented around chitosan, there is a chance for an over-exaggeration of its benefits. The accumulated documentation of the background science makes a strong case for the authenticity of chitosan, however negative results are not likely to pass peer review and remain unpublished. The reader is referred to recent, comprehensive reviews of plant applications [4,5] that list many purported beneficial applications of chitosan. The current review is a narrow focus on a few plant systems that delve deeply into the chitosan modes of actions that result in the induction of disease resistance responses in plant cells and suppress fungal spore growth.

1.1. *Chitosan source and chemistry*

Cellulose, chitin and chitosan are β-1,4-polymers of glucose, predominantly N-acetyl glucosamine and predominantly glucosamine, respectively (Fig. 1). These polymers are among the most prevalent found on the face of the earth [3]. Plants contain high percentages of cellulose but little or none of the glucosamine polymers. Cellulose is a formidable structural component of plants and chitin is a structural component of crustaceans, fungi and insects. Chitosan is found in walls of fungi. Plants have β-glucanase and chitinase the enzymes capable of digesting polymers of glucosamine and chitin respectively [6]. Since plants have the specific hydrolytic enzymes and are without the glucosamine polymers, these enzymes may have been retained throughout evolution for the purpose of confronting challenges by insects and fungi.

Chitin and chitosan are structural components of fungal cell walls. It was originally unexpected that the oligomers of these polymers can elicit defense responses and regulate other plant processes such as growth [7].

1.2. *Physical structure of chitosan*

The physical structures of both cellulose and chitosan are related by their strong β-1,4 linkages. Chitosan solutions can dry to form transparent sheets with the appearance of cellophane, a product of cellulose polymers. The chemical nature of chitosan differs from both chitin and cellulose because of the exposed amino groups that can bind positively charged protein molecules. Consequently, chitosan shares inductive properties with poly-cationic proteins such as protamine, histones and synthetic polymers of ornithine, arginine and lysine [8,9] but with out being digested by proteases.

1.3. *Molecular properties of chitosan*

Chitosan and chitin are not always pure polymers, chitosan often has ~20% of the N-acetyl glucosamine sugar residues of chitin and chitin often has ~20% of the glucosamine residues of chitosan. Both polymers are relatively water insoluble until broken down into oligomers with ≤7 sugar residues or 7 degrees of polymerization (dp) [10]. Longer polymers of chitosan are water soluble when in solutions at pH 7 or lower and thus are soluble in dilute organic acids such as lactic and acetic acids. The solubility can be maintained by slowly adjusting the solution back near pH 7 with NaOH. Chitosan should be in solution when evaluating the effects on plants. These physical properties of chitosan are obviously critical in the evaluation of the biological properties of both the parent polymers and the smaller oligomers. The marginal transfer of insoluble chitosan or its oligomers into a living cell would diminish or negate the cellular response. Such negative effects convert to skepticism of chitosan benefits. Chitosan molecules, as processed commercially, are high in molecular weight. When soluble, a portion of the molecule is taken up by plant cells. Chitinase is released by plant cells and may accommodate this transfer. Some chitinases [11] recognize the N-acetyl glucosamine linked regions of chitin and cleave within the 20% N-acetyl-glucosamine residue regions of chitosan molecules. Following cleavage the remaining polymer fragments would be reduced in size and enriched in glucosamine residues. With the controlled use of 6 N HCl or enzymatic digestion the long polymers can be fragmented chemically to produce shorter chitosan oligomers (~7 dp) that remain biological active and are directly soluble in water.

Chitosan gained world-wide attention because it could be derived from the waste products that remained in processing edible crustaceans. Marketable shrimp and crab meats are removed, leaving some residual meat, mineral and chitin [12]. The acetyl groups of chitin are removed with a high molarity base [5]. Chitosan is maximally viscose when prepared with high degrees of polymerization and in high concentration. Purified chitosan is a suitable resource molecule for numerous derivitizations and thus polymer chemists have found uses for both chitosan and modified chitosan.

2. Chitosan in plant defense

2.1. *Plant cellular responses to chitosan*

The reported biochemical and molecular changes in plants treated with chitosan include: DNA damage, chromatin alterations[13,14], increases in cytosolic Ca^{2+}[15], activation of MAP-kinases [16], oxidative burst [17], callose apposition[18], increase in pathogenesis-related (PR) gene mRNA, PR protein synthesis [19], phytoalexin accumulation, hypersensitive response (HR) [20], and in some systems, synthesis of jasmonic acid (JA) and abscissic acid (ABA) and accumulation of hydrogen peroxide [21,22].

2.2. *The pea plant defense model: pea endocarp/Fusarium solani fungi*

Chitosan has been analyzed extensively in a pea endocarp tissue test system. This cuticle-free, inner surface of an immature