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Short Communication

CHITOSAN - Molecular Forms with Potential in Agriculture and Medicine

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Abstract

Chitosan has been utilized effectively in many plant-related applications since becoming a commercial product. The multiple forms of chitosan represent many physical and biological properties. Based on the current paucity of reports on chitosan oligomers in animal systems, the utility of these properties are likely new to many researchers. The objective of this report is to assemble some basics of size, cationic and solubility properties of chitosan relative to function and encourage further research on the chitosan heptamer as a gene activating signal in animal tissue. Also to compliment other work reported in the special report on chitosan.

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- Gene activation in animal tissue

INTRODUCTION

The information on the molecular forms of chitosan in this communication is designed to complement other reports associated with the regular edition on "chitosan". Chitosan is a naturally occurring compound. A typical commercial source is from the chitin within crustacean shells. The commercial processes for its production and for use in plant systems [1-3] are published. There are different innovative uses for the multiple molecular forms of chitosan. The value of each form depends on understanding that the molecular properties change: Such as the molecular size, degrees of polymerization, cationic properties and water solubility. The chitosan source typically initiates as a chitin precursor in the by-product of shrimp or crab shell seafood industry. The shell waste is deproteinized and the chitin within is deacetylated to chitosan. The final chitosan product is usually 80% deacetylated and consists of a high molecular weight polymer of mostly glucosamine linked β-1,4. The remaining 20% consists of acetylated glucosamine and retains some chitin-like properties.

The objective of this short communication is to give examples of beneficial chitosan uses based both on polymer size and biochemistry and encourage its further use in medicine as a chromatin associating component. The values of this unique and potentially abundant molecule are re-emphasized, but now with additional attention to a chitosan oligomer, the heptamer, that is biologically dynamic. The chitosan oligomers have been somewhat passed over by research favoring the larger less expensive high molecular weight forms. There are many biochemical and transcriptional functions that remain to be discovered and pursued relative to these smaller oligomers (short polymers of glucosamine).

Long chain chitosan

First, some applications benefit from the long viscous chains of polymerized glucosamine, the typical initial product developed following the de-acetylation of chitin. The amino groups on glucosamine polymers are exposed alternately along the chain. Their positively charges develop strong affinities for negatively charged molecules of any type. Chitosan is useful for aggregating material in sewage or constituents of our dietary tracks consequentially enabling waste processing or preventing the utilizations of the part of consumed food, respectively. Thus, there are physical roles for chitosan in waste removal and some utility for weight reduction in humans [4].

Following the removal of water from chitosan solutions a transparent layer resembling cellophane remains. Dental surgeons over-lay these flat sheets to assist wound healing following surgical incisions [5]. As a result, incisions are held in place during healing and some inherent hydrolytic enzymes can harmlessly remove the chitosan patch over time.

Long chain chitosan is a part of a protective procedure for reducing the destruction of potatoes by the Late Blight-inciting organism, *Phytophthora infestans* [6]. Although "chitosan only" individual applications can induce a defense response in potatoes, the long strands of chitosan infiltrated with a strong anti-fungal compound, such as copper sulfate pentahydrate, provides substantially more protection at the leaf surface against Potato Late Blight. The strategic dispersal on the leaf top of the copper compound by chitosan reduces the application rate by 40 times the level required if the copper is applied alone.

The "stick to anything" property of chitosan has been

resourcefully utilized to remove toxic, negatively-charged dyes or other components from water suspensions [7]. The pre-sticking of chitosan to a dense material such as sand causes rapid sedimentation. A similar chitosan-sand-copper sulfate pentahydrate combination was used to selectively sediment the herbicidal copper compound onto milfoil plants for eradicating-action towards the invasive weed species, Eurasian Milfoil, from deep water (Figure 1) (Hadwiger unpublished).

General solubility properties of chitin and chitosan

The final synthetic production step from chitin to chitosan is often catalyzed with a strong base. The generated chitosan is insoluble and sediments. Chitosan is not water soluble at basic pH, but is soluble in dilute organic acids such as lactic or acidic acid. The pH can be readjusted with base to ~6.5 pH and retain solubility. The β-1,4 link of glucosamine sugars can be attacked with 6 N HCl and can result in an array of oligomers with varying degrees of polymerization (dp). The water solubility of these oligomers improves from 10 dp down to the free glucosamine sugar. D-glucosamine oligosaccharides can also be produced by the enzymatic hydrolysis of chitosan [8].

Short chain oligomers of chitosan

The biological properties of chitosan oligomers in plant and fungal systems have been examined previously [9,10]. The oligomer that optimally induces defense responses in plants is the heptamer. This size is also optimal for inhibiting microbial growth. The Hadwiger laboratory first observed chitosan's ability to induce the activity of a secondary pathway in plants that culminates in the accumulation of the phytoalexin, pisatin, an antifungal isoflavonoid [11] and to be effective in directly inhibiting the growth of some plant pathogenic fungi [12].

To confirm that the biological form possessed the proper degree of polymerization (dp) the chitosan oligomer was organically-synthesized [10]. Computer-generated models suggested that a 7 or 8-mer may preferentially reside in the minor groove of the DNA molecule [3]. The chitosan oligomers below 5 dp were biologically less effective. The poly-glucosamine heptamer was capable of inducing a completely effective defense response in pea tissue against a true pathogen of pea. This chitosan oligomer action was associated with the activation of defense genes called pathogenesis-related (PR) genes. These response genes possess abilities to slow or stop the growth of fungi [13]. We surmised that the action of chitosan oligomers in triggering the PR response, were directly on the DNA of plant chromatin, since the 2D electrophoresis separation of *in vitro*-translated proteins coded by defense-response-related RNAs were similar to the proteins generated by chitosan and from more authenticated DNA specific molecules such as actinomycin D. Actinomycin D is a DNA intercalator [14]. The chitosan DNA-targeting was further verified by the finding that 19% of the radio-labelled chitosan entering the plant cell was recoverable in the plant nucleus [3].

Figure 1 Effect of a chitosan composite (chitosan 0.12%; copper sulfate pentahydrate 0.004%; sand 0.983 %) on Eurasion water milfoil. One gram of the composite was applied at the top of the container for 1 min. and the propagules with the sedimented composite attached were then transferred to new growth media. The left photo control remained alive at 15 days and the two treatments with chitosan and copper sulfate pentahydrate (middle and right photo) died.

The chitosan label also accumulates in the membrane/wall region of the plant cell which suggests alternative modes of action such as the generation of reactive oxygen species (ROS) that in turn could be signaling defense gene activation. In pea endocarp tissue the ROS, hydrogen peroxide is generated by fungal spores and dissipates within 1 hour. When hydrogen peroxide is applied externally it is unable to induce significant levels of phytoalexin that is a part of the defense response triggered by spores [15]. The basis of chitosan's antimicrobial activity has been related to membrane damage, chelation action and inhibition of DNA and RNA synthesis targeting the fungal nucleus [16].

Chitosan and chitosan oligomers in animal cells

To date, the chitosan oligomers have been under-utilized in inducing defense processes in animal cells. Chitosan has been used as a carrier for DNA segments that contain gene constructs for transforming plant cells, demonstrating that chitosan can traverse cell membranes. Other work has shown that chitosan may have a role in stabilizing drugs or transporting them through internal barriers. In rat and mice chitosans displayed no anti-inflammatory properties but showed good anti-ulcerative and wound healing abilities [17]. Chitosan also reduced ulcerative wounds and helped retain the stomachal fold rendering a gasto protective effect. However, the prospects for its future uses involving gene regulation in animal systems appears to have been largely overlooked. Chitosan oligomers have been reported to both up-regulate pro-apoptotic protein Bax, triggering the start-up of an apoptotic program [18] and positively affect inflammatory bowel disease through inhibition of NF-kB signaling and apoptosis of intestinal epithelial cells [19]. The oligomers are known to have anti-microbial properties that interfere with RNA synthesis in fungi [13]. Anti-tumor and anti-cancer activity also appears to be due to the cationic property exerted by the amino groups [20]. This connection has yet to be associated in animal tissue with the affinity of chitosan oligomers to the DNA of chromatin as has been articulated for plant defense mechanisms [21,22]. There is a recognition in animal systems, that immune-stimulating properties of chitosan oligomers may be responsible for antitumor activity [23]. Even though there is the possibility that all of these changes culminate in the orderly progression to apoptosis in animal cells, the development of "nonhost disease resistance" in plants has been shown to occur hours before cell death [24] is detectable. The strong affinity of chitosan for DNA *in vitro* provides support for direct *in vivo* interaction with nuclear DNA in competition with nuclear proteins such as HMG A and histones H2A and H2B [22]. This direct route may be alternative to signaling routes via phosphorylation cascades etc. Chitosan oligomers appear to be capable of by-passing the longer route of membrane-specific reception. The short chain features of the chitosan heptamer and the calculated potential of it to reside in the minor groove of the DNA open prospects for numerous chromatin changes including DNA torsional change, strand breakage, histone exclusion and histone competition all of which can effect "stalled RNA polymerase complexes" [25]. Hypothetically it is these chromatin changes [22] that release such complexes enabling the transcription of plant defense genes. Since some fungi that infect animals also possess cell walls and contain both chitin and chitosan, the presence of fragments (oligomers) of digested chitin/chitosan could be expected to mimic plant/fungal interactions [9] in releasing such chitin and chitosan oligomers.

CONCLUSIONS

Hopefully, this short communication will encourage the interests of animal labs to encompass research that considers the feasibility that a direct interaction between chitosan oligomers and animal nuclei may provide answers to the current lack of understanding of chitosan's anti-inflammatory and other cellular properties.

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REFERENCES

1. Sandford PA. 'Chitin and Chitosan', (Eds G. Skjak-Braek, T. Anthonsen and P. A. Sanford) Elsevier Applied Science, London. 1989; 51.
2. Hadwiger LA. Multiple effects of chitosan on plant systems: Solid science or hype. Plant Sci. 2013; 208: 42-49.
3. Hadwiger LA, Chiang C, Victory S, Horovitz D. The molecular biology of chitosan in plant/pathogenic interaction. In: 'Chitin and chitosan' (Eds G. Skjak-Braek, T. Anthonsen and P. A. Sanford) Elsevier Applied Science, London. 1989; 11.
4. Mhurchu CN, Dunshea-Mooij C, Bennett D, Rodgers A. Effect of chitosan on weight loss in overweight and obese individuals: A systematic review of randomized controlled trials. Obes Rev. 2005; 6: 35-42.
5. Muzzarelli R, Biagini G, Pugnaloni A, Filippini O, Baldassarre V, Castaldini C, et al. Reconstruction of parodontal tissue with chitosan. Biomaterials. 1989; 10: 598-603.
6. Hadwiger LA, McBride PO. Low-level copper plus chitosan applications provide protection against late blight of potato. Plant health Progress. 2006.
7. Crini G, Badot PM. Application of chitosan, a natural aminopolysacccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: A review of recent literature. Prog Polym Sci. 2008; 33: 399-447.
8. Izume M, Ohtakara A. Preparation of D-glucosamine oligosaccharides by the enzymatic hydrolysis of chitosan. Agric Biol Chem. 1984; 51: 1169-1191.
9. Kendra DF, Hadwiger LA. Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum*. Exp Mycol. 1984; 8: 276-281.
10. Hadwiger LA, Ogawa T, Kuyama H. Chitosan polymer sizes effective in inducing phytoalexin accumulation and fungal suppression are verified with synthesized oligomers. Mol Plant-Microb Interact. 1994; 7: 531-533.
11. Hadwiger LA, Beckman JM. Chitosan as a Component of Pea-Fusarium solani Interactions. Plant Physiol. 1980; 66: 205-211.
12. Allan CR, Hadwiger LA. The fungicidal effect of chitosan on fungi of varying cell wall composition. Mycology. 1979; 3: 285-287.
13. Hadwiger LA, Kendra KF, Fristensky BW, Wagoner W. Chitosan both activates genes in plants and inhibits RNA synthesis in fungi. In: Chitin in Nature and Technology, Ed. Muzzarelli, R. Plenum Press N/Y. 1986; 209-214.

14. Loschke DC, Hadwiger LA, Wagoner W. Comparison of mRNA populations coding for phenylalanine ammonia lyase and other peptides from pea tissue treated with biotic and abiotic phytoalexin inducers. Physiol Plant Pathology. 1983; 23: 163-173.

15. Tanaka K, Hadwiger LA. Nonhost resistance: Reactive oxygen species (ROS) signal causes DNA damage prior to the induction of PR genes and disease resistance in pea tissue. Phys Molec Plant Pathology. 2017; 98: 18-24.

16. Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W. Chitosan as antimicrobial agent: Applications and mode of action. Biomacromolecules. 2003; 4: 1457-1465.

17. Tavaria K, Freni P, Michelle PJ, Ruiz LTG, Ana E, Manuela P, et al. Anti-proliferative, anti-inflammatory, anti-ulcerogenic and wound healing properties of chitosan. Current Bioactive Compounds 2016; 12:114-122.

18. Xu Q, Dou J, Wei P, Tan C, Yun X, Wu Y, et al. Chitooligosaccharides induce apoptosis of human hepatocellular carcinoma cells via up-regulation of Bax. Carbohydrate Polymers. 2008; 71: 509-514.

19. Yousef M, Pichyangkura R, Soodvilai S, Chatsudthipong V, Muanprasat C. Chitosan oligosaccharide as potential therapy of inflammatory bowel disease: Therapeutic efficacy and possible mechanisms of action. Pharmacol Res. 2012; 66: 66-69.

20. Qin C, Du Y, Xiao L, Li Z, Gao X. Enzymic preparation of water-soluble chitosan and the antitumor activity. Internat J Biol Macromol. 2002; 31: 111-117.

21. Hadwiger LA. Anatomy of a nonhost disease resistance response of pea to *Fusariumsolani*: PR gene elicitation via DNase, chitosan and chromatin alterations. Front Plant Sci. 2015; 6: 373-400.

22. Isaac J, Hartney SL, Druffel K, Hadwige LA. The nonhos disease resistance response in peas; alterations in phosphorylation and ubiquitination of HMG A and histones H2A/H2B. Plant Science. 2009; 177: 439-449.

23. Suzuki K, Mikami T, Okawa Y, Tokoro A, Suzuki S, Suzuki M. Antitumor effect of hexa-N-acetylchitohexaose and chitohexaose. Carbohydr Res. 1986; 151: 403-408.

24. Choi JJ, Klosterman SJ, Hadwiger LA. A comparison of the effects of DNA damaging agents and biotic elicitors on the induction of plant defense genes, nuclear distortion and cell death. Plant Physiol. 2001; 125:752-762.

25. Wu JQ, Snyder M. RNA polymerase II stalling: loading at the start prepares genes for a sprint. Genome Biol. 2008; 9: 220-227.

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