Journal of Controlled Release 134 (2009) 158–168

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Review

Nucleic acid delivery with chitosan and its derivatives

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ARTICLE INFO

Article history:
Received 25 June 2008
Accepted 11 November 2008
Available online 30 November 2008

Keywords:
Chitosan
Delivery
Non-viral vectors
Nucleic acid
Transfection efficiency

ABSTRACT

Chitosan is a naturally occurring cationic mucopolysaccharide. It is generally biocompatible, biodegradable, mucoadhesive, non-immunogenic and non-toxic. Although chitosan is able to condense nucleic acids (NA) (both DNA and RNA) and protect them from nuclease degradation, its poor water solubility and low transfection efficacy have impeded its use as an NA carrier. In order to overcome such limitations, a multitude of strategies for chitosan modification and formulation have been proposed. In this article, we will first give a brief overview of the physical and biological properties of chitosan. Then, with a special focus on plasmid DNA delivery, we will have a detailed discussion of the latest advances in chitosan-mediated NA transfer. For future research, the following three important areas will be discussed: chitosan-mediated therapeutic small RNA transfer, structure–activity relationships (SAR) in chitosan vector design, and chitosan-mediated oral/nasal NA therapy.

Contents

1. Introduction

Nucleic acid (NA) therapy is an emerging field in medical and pharmaceutical sciences due to its potential for treating a variety of genetic disorders that have been incurable to date. NA therapy consists of two parts: First, the therapeutic NA itself, including plasmid DNA (pDNA) and different types of small RNA, and second, the NA carrier, including viral and non-viral vectors.

From the last century onwards, pDNA encoding therapeutic genes have been extensively studied in NA therapy. Those encoded genes include metabolic suicide genes (e.g. herpes simplex thymidine kinase [1], varicella zoster thymidine kinase [2] and *Escherichia coli* cytosine deaminase [3]), and genes encoding the following proteins: angiogenesis inhibitors, proapoptotic proteins, pro-drug activators or immune response modulators. In addition to pDNA, small RNA like micro-RNA (miRNA), small interfering RNA (siRNA) and antisense RNA have also been widely explored as tools for genetic treatments.

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0168-3659/$ – see front matter
doi:10.1016/j.jconrel.2008.11.021

Due to the low transfection efficiency of naked NA injections *in vitro* and *in vivo*, many NA delivery vehicles have thus been investigated. In 2003, China was the first country to approve clinical uses of an adenoviral vector carrying the p53 gene in therapy for head and neck squamous cell carcinoma (HNSCC) [4]. Though viral vectors have the merit of high transfectability, their potential safety risks [5,6] as well as immunogenicity warrant the search for alternative non-viral vectors [7]. In 1988, a water soluble polymeric DNA carrier consisting of a galactose-terminal (asialo-) glycoprotein (called asialoorosomucoid) covalently linked to poly(L-lysine) (PLL) was reported [8]. Since then, copious other polymeric NA carriers have been explored, which include chitosan, polyethylenimine (PEI) [9–12], PLL, poly(lactide-co-glycolide) (PLGA) [13], and polypropylenimine dendrimers [14]. In this article, we will narrow down the discussion to chitosan. With a special focus on pDNA delivery, we will first have a critical review of the latest advances in chitosan-mediated NA transfer, followed by a prospective discussion of a number of areas that are noteworthy in future chitosan-mediated NA delivery research.

2. Plasmid DNA delivery with chitosan and its derivatives

2.1. Overview of chitosan

Chitosan is a linear polysaccharide composed of randomly distributed *N*-acetyl-D-glucosamine and β-(1,4)-linked D-glucosamine. Major techniques for its structural characterization include potentiometry [15], infrared spectroscopy [16], ultra-violet spectroscopy [17] and ^{1}H nuclear magnetic resonance (^{1}H NMR) [18]. By using the last method, we have reproduced the NMR spectrum of chitosan as shown in Fig. 1. Commercially, chitosan can be obtained from chitin via alkaline deacetylation, which is performed by decolorizing crab and shrimp shells with potassium permanganate and then boiling them in sodium hydroxide [19].

In the physiological environment, chitosan can be digested by lysozymes or certain bacterial polysaccharide-degrading enzymes produced by the intestinal flora [20,21]. Because of its biodegradability, biocompatibility, non-allergenicity and NA binding ability, chitosan has been widely studied as a pDNA carrier since the beginning of the last century. In general, chitosan-based pDNA transfer depends greatly on both the ionic and non-ionic interactions between the carbohydrate backbone of chitosan and surface proteins of transfected cells [22]. Like many other polymeric vectors, chitosan-mediated pDNA delivery may involve a number of cellular barriers [11,23,24], including enzymatic degradation, inefficient cellular uptake, encapsulation in endo-lysosomes, failure of NA/polymer dissociation, and nuclear localization.

Compared with PEI (another well-studied polymer with generally high transfection efficiency but significant cytotoxicity), chitosan has lower transfectability. This is believed to be related to its comparatively weak endo-lysosomalytic proton sponge effect. Such postulation is supported by the differences in buffering capacities of PEI and chitosan as shown by our performed acid–base titration (Fig. 2). Nevertheless, chitosan has been recognized as a membrane perturbant. By studying influences of pH and chitosan molecular weight on 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) bilayer–chitosan interaction, Fang et al. discovered the effects of chitosan on membrane perturbation, and associated the magnitude of this effect with the degree of protonation on chitosan [25]. They have also reported that an increase in the chitosan mole fraction can lead to a significant reduction in cooperative units against molecular weight (M.W.), implying that chitosan can swirl across the membrane lipid bilayer and facilitate the cellular uptake of the polyplex.

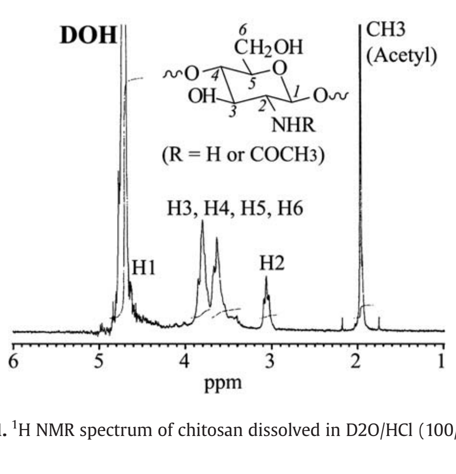

Fig. 1. ^{1}H NMR spectrum of chitosan dissolved in D2O/HCl (100/1 v/v).

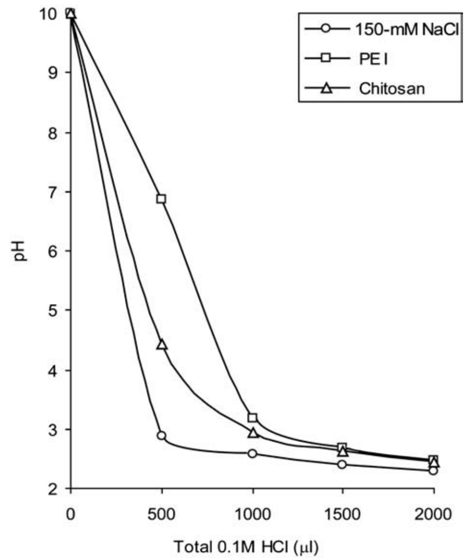

Fig. 2. Acid–base titration profiles of 150 mM NaCl, PEI (600Da) and chitosan. The 150 mM NaCl solution was used to dissolve both PEI and chitosan prior to the measurement.

Chitosan has primary amine groups with a pKa value of approximately 6.5 [26]. It forms positively charged single helicoidal stiff chains in acidic aqueous medium [27]. The positive surface charge of chitosan allows it to interact with macromolecules like exogenous NA, negatively charged mucosal surfaces, or even the plasma membrane [28]. Moreover, the configuration of chitosan is fully displaced under acidic pH, and it is in this configuration that chitosan can trigger the opening of tight junctions, and hence enhance the paracellular transport of hydrophilic agents [29].

2.2. Uses of chitosan in plasmid DNA transfer

Mumper et al. was the first team, to our knowledge, to recognize and study the potential of chitosan for *in vitro* pDNA delivery [30]. By varying the N/P ratio (the ratio of chitosan nitrogens to DNA phosphates) of the complex coacervate and the molecular weight of chitosan used, polyplexes (mean sizes ranged from 150–600 nm) with different topological conformations including spherical, annular [31], toroidal [31,32] and globular [31,33] morphologies are observed.

Practically, chitosan particles can be prepared by different methods including coacervation [34], ionic gelation [35], covalent cross-linking [36] and desolvation [37]. The variation in preparation techniques together with other parameters like NA concentrations, salt concentrations, pH, charge ratios and coacervation temperatures can influence the size of the chitosan–DNA complex produced, and hence the overall transfection efficacy [38–41]. This is illustrated by the observation of enhanced transfectability of chitosan in highly differentiated cystic fibrosis bronchial epithelial CFBE4o- cells under acidic medium [42]. Such increase in transfection is also assumed to be