Carbohydrate Polymers 80 (2010) 285–290

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Development and characterization of self-aggregated nanoparticles from anacardoylated chitosan as a carrier for insulin

R. Shelma, Willi Paul, Chandra P. Sharma *

Division of Biosurface Technology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram 695012, India

ARTICLE INFO

Article history:
Received 15 September 2009
Received in revised form 12 November 2009
Accepted 18 November 2009
Available online 22 November 2009

Keywords:
Nanoparticles
Self-aggregation
Chitosan
Anacardic acid

ABSTRACT

Nanoparticulate carriers made from biodegradable polymers especially from chitosan seems to be an excellent approach to increase the uptake and transport of orally administered insulin. Various approaches have been studied to develop nanoparticles from chitosan including self-aggregated nanoparticles from hydrophobically modified chitosan. Anacardic acid is a naturally occurring fatty acid having bulky aromatic group as well as long aliphatic chain and an amphiphilic monomer of great potential. An attempt has been made to develop and characterize self-aggregated nanoparticles from chitosan modified with anacardic acid. Anacardoylated chitosan spontaneously formed nanoparticles in aqueous insulin solution with a particles size of 214 nm diameter at neutral pH. The hydrophobic nature of the nanoparticles helped in the sustained release of insulin in the intestinal environment and the released insulin was stable and retained its conformation. However, it released insulin in acidic conditions and need to be encapsulated in alginate to render pH sensitiveness.

1. Introduction

Injections had been the only available route for the delivery of insulin since its discovery by Banting and Best in 1921. Oral delivery of insulin can replace daily injections to diabetic patients, however, poses unique problem of stability and susceptibility to proteolysis, which reduce their bioavailability. Administration of therapeutic peptide drug such as insulin via oral route represents one of the greatest challenges in modern pharmaceutical technology (Morishita & Peppas, 2006). The protection of insulin against self-aggregation as well as enzymatic degradation is an important issue for an oral insulin delivery system (Hega et al., 1990). Successful systemic uptake after oral delivery is difficult to achieve because insulin is a large molecule and hydrophilic with possible enzymatic degradation before reaching the site of absorption (Lee & Yamamoto, 1990). Nanoparticulate carriers made from biodegradable polymers seems to be an excellent approach to increase the uptake and transport of orally administered insulin. Like sub-micron emulsions and liposomes, biodegradable polymeric nanoparticles offer a higher stability when they are in contact with biological fluids (Hans & Lowman, 2002). The polymeric nature of nanoparticles protects the drug from adverse external conditions and controls its release (Calvo, Alonso, Vila-Jato, & Obinson, 1996; Romero-Cano & Vincent, 2002; Sakuma et al., 1997; Takeuchi, Yamomoto, & Kawashima, 2001). Moreover, it has been shown that M-cells located on the surface of Peyer's patches are a possible pathway for transporting the nanoparticles through the epithelium of the gut (Clean et al., 1998; Jung et al., 2000). There is a challenge in encapsulating water soluble drugs in water insoluble polymers efficiently (Niwa, Takeuchi, Huno, Kunuo, & Kawashima, 1994; Park, Lu, & Crotts, 1995). Various reports on investigations to improve the oral bioavailability of insulin suggests delivering insulin utilizing nanoparticles made from chitosan or chitosan derivatives and other bioadhesive polymers (Pan et al., 2002).

Interest in chitosan for pharmaceutical formulation continues to grow. Chitosan is a unique polymer that has demonstrated utility in a number of applications for oral drug delivery. Chitosan's film forming ability makes it a coating agent for conventional solid dosage forms such as tablets. Moreover, its gel and matrix forming abilities makes it useful for solid dosage forms such as granules, microparticles, etc. Chitosan also serve as a controlled-release matrix, in addition to properties such as mucoadhesion and permeation enhancement due to its cationic nature which is capable of opening tight junctions in a cell membrane to improve oral bioavailability of a drug. Nanoparticles are normally utilized for drug delivery applications because of its high stability, prolonged residence time, high drug encapsulation, better storage life and the ability to translocate through the intestinal barrier; by Para cellular pathway or via M-cells in Peyer's patches (Hussain, Jaitley, & Florence, 2001).

Anacardic acid is a natural fatty acid having bulky aromatic group as well as long aliphatic chain, found in the exudates of

* Corresponding author. Tel.: +91 471 252 0214; fax: +91 471 234 1814.
E-mail address: sharmacp@sctimst.ac.in (C.P. Sharma).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.11.025

plants which belong to Anacardeacea family. Moreover, it is a naturally occurring biocompatible amphiphilic monomer of great potential. It is a natural salicylic acid derivative with an unsaturated non-isoprenoid long aliphatic side chain. It is also a natural aspirin derivative which has shown bioactivity (Acevedo et al., 2006; Balasubramanyam, Swaminathan, Ranganathan, & Kundu, 2003; Bhattacharya, Mukhopadhyay, Rao, Bagchi, & Ray, 1987; Pillai, 1997). Various reports on chitosan have shown that control of drug release was improved by hydrophobic stabilization of matrices and substitution degree (Kurita, 2001; Martin et al., 2002; Noble, Gray, Sadiq, & Uchegbu, 1999). In the present study an attempt has been done to modify chitosan region-specifically by *N*-acylation with anacardic acid, a natural salicylic acid derivative which is biocompatible; to develop self-aggregates of hydrophobically modified chitosan through acid chloride route as a matrix for oral delivery of insulin. Nanoparticles with an average size of about 214 nm diameter were developed without any loss of conformational variation and aggregation of encapsulated insulin. The nanoparticles were further encapsulated in sodium alginate for obtaining pH sensitiveness to the final formulation.

2. Materials and methods

Chitosan (degree of deacetylation of 86% with a molecular weight of 196 kDa) was obtained as a gift from India Sea Foods Pvt. Ltd., Cochin, India. Anacardic acid (AA) was obtained as a gift from Adarsh Industries, Karnataka, India. Acetic anhydride was from Merck KGaA, Darmstadt, Germany. Thionyl chloride and pyridine were from SD Fine Chem India Ltd., Mumbai, India. Insulin (human, 400 IU/ml) was a gift from USV Ltd., Mumbai, India. The chemicals and other solvents used were of analytical reagent grade.

Anacardoylation of chitosan was done in two steps. In the first step anacardic acid was acetylated by heating a mixture of 12 g anacardic acid and 36 g acetic anhydride under stirring for 3 h in an oil bath at 80 °C. After the reaction the mixture was cooled to room temperature. This was precipitated in cool distilled water. The precipitate obtained was then extracted in ether. The ethereal layer was separated using a separating funnel and dried to obtain acetylated anacardic acid. In the second step the acetylated anacardic acid was stirred with 10 ml of thionyl chloride in the presence of a drop of pyridine for 2 h at 60 °C in an oil bath. Excess thionyl chloride was allowed to evaporate by raising the temperature to 80 °C. To this 10 ml of dimethyl formamide and 2 g of chitosan was added and stirred for 16 h at room temperature. This was precipitated in distilled water, washed three times with distilled water and dried to obtain anacardoylated chitosan. Anacardoylated chitosan was characterized by FTIR spectroscopy utilizing a Nicolet Impact 410 FTIR spectrometer. For NMR measurements, the sample was dissolved in D_2O acidified with acetic acid, freeze dried to displace adsorbed moisture, and then dissolved in the same solvent. The sample concentration was 10 mg/ml in D_2O (99.9%). ^1H NMR was carried out in a Bruker spectrometer with 500 MHz.

Specific amounts of anacardoylated chitosan were dissolved in DMF to obtain three different concentrations of anacardoylated chitosan. This was added to the insulin solution (400 IU/ml) to obtain insulin-loaded chitosan self-aggregated nanoparticles. This was centrifuged at 10000 RPM for 10 min and the pellet obtained was dried in a refrigerator at 4 °C. Chitosan nanoparticles as a control were prepared by mild ionotropic gelation with sodium tripolyphosphate as per standard reported procedure (Fernández-Urrusuno, Calvo, Remuñán-López, Vila-Jato, & Alonso, 1999). The particle size and zeta potential and the pH titrations of these self-aggregated nanoparticles were analyzed using a zetasizer, Nano ZS and MPT-2 autotitrator (Malvern Instruments Limited, UK).

The in vitro cytotoxicity of the nanoparticles was evaluated by MTT assay (Mosmann, 1983) done on mouse fibroblast (L929) cell lines as per the directions of ISO standard (ISO, 1999).

Encapsulation efficiency and insulin loading of the nanoparticles were evaluated. A known quantity of insulin loaded nanoparticles was incubated at 30 °C in a known quantity of phosphate buffer (pH 7.4) for 24 h. This was filtered using a 0.4 μm syringe filter and the insulin content estimated. The insulin content was estimated by evaluating the protein content by Lowry's method at 750 nm using UV spectrophotometer (UV l60A, Shimadzu). The insulin content was also estimated using RIA which also indicates its immunoreactivity. RIA was performed following the Coat-A-Count Protocol. The radioactivity was measured by counting the tubes in a gamma counter (1470 Automatic Gamma Counter, Perkin Elmer Wizard).

The release of insulin from the nanoparticles was carried out in simulated gastric (SGF, pH 1.2) and intestinal (SIF, pH 6.8) fluids. SIF and SGF were prepared fresh in the laboratory with the composition as per USP without the addition of enzymes (The United States Pharmacopeia, USP 28, 2005, United States Pharmacopeial Convention Inc., Rockville, MD, USA, pp. 2855–2858). The nanoparticles (10 mg) were introduced into 5 ml of SGF and SIF, respectively, in a screw-capped bottle under sterile conditions. Aliquots of 0.5 ml were withdrawn at various time intervals and insulin content was estimated as described earlier. An equal amount of respective buffers was added in order to maintain a constant volume. To bypass the hostile environment of the gastric region these nanoparticles were encapsulated in calcium alginate. The drug loaded nanoparticles (100 mg) were mixed with 2 ml of 2% sodium alginate solution and added drop wise into 2% calcium chloride gelling bath using a syringe. The capsules obtained were rinsed with water and dried at 4 °C in a refrigerator. The dry particles obtained were in the range of 100 μm with a final weight of 140 mg.

The supernatant of the released insulin solution was concentrated to 50 IU/ml (2 mg/ml approx) using a stirred cell, attached with an ultrafiltration filter of 5 kDa cutoff (Millipore, Model 8050) which is generally used for protein purification. CD spectra at 25 °C were acquired using a Jasco J-810 spectropolarimeter as reported (Ramachandran, Paul, & Sharma, 2009).

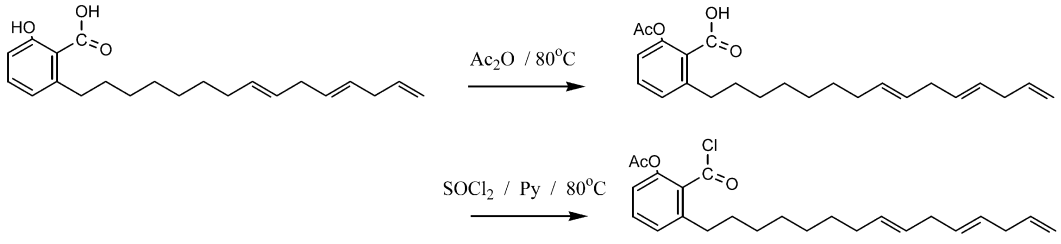

Fig. 1. Scheme of acid chloride reaction with anacardic acid.