Nanoparticulate Assembly of Mannuronic Acid- and Guluronic Acid-Rich Alginate: Oral Insulin Carrier and Glucose Binder

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ABSTRACT: The relationship of high and low molecular weight mannuronic acid (M)- and guluronic acid (G)-rich alginate nanoparticles as oral insulin carrier was elucidated. Nanoparticles were prepared through ionotropic gelation using Ca^{2+}, and then *in vitro* physicochemical attributes and *in vivo* antidiabetic characteristics were examined. The alginate nanoparticles had insulin release retarded when the matrices had high alginate-to-insulin ratio or strong alginate–insulin interaction via O–H moiety. High molecular weight M-rich alginate nanoparticles were characterized by assemblies of long polymer chains that enabled insulin encapsulation with weaker polymer–drug interaction than nanoparticles prepared from other alginate grades. They were able to encapsulate and yet release and have insulin absorbed into systemic circulation, thereby lowering rat blood glucose. High molecular weight G- and low molecular weight M-rich alginate nanoparticles showed remarkable polymer–insulin interaction. This retarded the drug release and negated its absorption. Blood glucose lowering was, however, demonstrated *in vivo* with insulin-free matrices of these nanoparticles because of the strong alginate-glucose binding that led to intestinal glucose retention. Alginate nanoparticles can be used as oral insulin carrier or glucose binder in the treatment of diabetes as a function of its chemical composition. High molecular weight M-rich alginate nanoparticles are a suitable vehicle for future development into oral insulin carrier. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:4353–4363, 2013
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INTRODUCTION

Alginate, a water-soluble polysaccharide, is commonly isolated from brown algae, such as *Laminaria hyperborea*, *Ascophyllum nodosum*, and *Macrocystis pyrifera*.[1,2] Its chain is made of homopolymeric regions of β-D-mannuronic acid (M) blocks and α-L-guluronic acid (G) blocks, interdispersed with alternating α-L-guluronic and β-D-mannuronic acid blocks (Fig. 1). The viscosity, binding affinity for cations, gelation, aqueous solubility, mechanical strength, swelling capacity, and bioadhesiveness of alginate are dictated by its uronic acid composition and molecular weight.[2–8] The viscosity of alginate increases with its molecular weight and is a function of polymer conformation. The mannuronate unit is reported to have a lower affinity for calcium ion (Ca^{2+}) than guluronate moiety.[1,2]

Alginate is a nontoxic, biodegradable, nonimmunogenic, and biocompatible polymer.[5,9–13] It possesses several inherent biological effects such as anticholesterolaemic, antihypertensive, antidiabetic, antiobesity, antimicrobial, anticancer, antihepatotoxicity, wound healing, anticoagulation, and coagulation activities.[2] The biological effects of alginate have been indicated to be attributed to its structural assembly and physicochemical features. The depolymerized or low molecular weight alginate has been found to be able to control the development of diabetes. Diabetes mellitus is an endocrine disease associated with the disorders of carbohydrate metabolism brought about by deficiency in insulin secretion, insulin resistance, or both.[14,15] The epidemiology study indicates that hyperglycemia is the primary cause of diabetes. The primary mode of treatment of type I diabetes is exogenous insulin administration by means of subcutaneous route. Following chronic subcutaneous insulin administration, lipoatrophy or lipohypertrophy, however, tends to surface at the injection sites. The injection mode has also brought about pain, hypoglycemia, and risk of infection at injection site.

Oral administration of insulin has been the alternative goal of many researchers.[14,15] Lately, polymeric nanoparticles have been decorated with pH-sensitive moiety, bioadhesive, and/or water-insoluble materials to promote insulin delivery in gastrointestinal tract.[15] The overview of various strategies applied in design of oral insulin nanoparticles denotes the significance of mucosa adhesiveness of drug delivery system. A prolonged adhesion of dosage form in the intestinal tract could translate to cumulative release and absorption of insulin transcellularly, paracellularly, and/or via the Peyer's patches. It overcomes the absorption limit of insulin. Nanoparticles, being small in physical dimension, exhibit a large specific surface area. Their nanometric feature enhances particulate mucoadhesion and facilitates oral insulin delivery. The latter can also be promoted through sustaining drug release from nanoparticles to allow timely insulin absorption without premature drug contact with the gastrointestinal milieu.[16]

Low molecular weight alginate has been widely used as the matrix substance of oral insulin nanoparticles.[17–20] Though low molecular weight alginate inherently possesses antidiabetic activity,[2] the matrix made of such polymer releases drugs at a fast rate.[5] Alginate's uronic acid composition can critically affect drug release characteristics of a matrix as a function of matrix pore, crack, lamination, and diffusion barrier formation.[2,21] The construction of oral insulin nanoparticles has nonetheless

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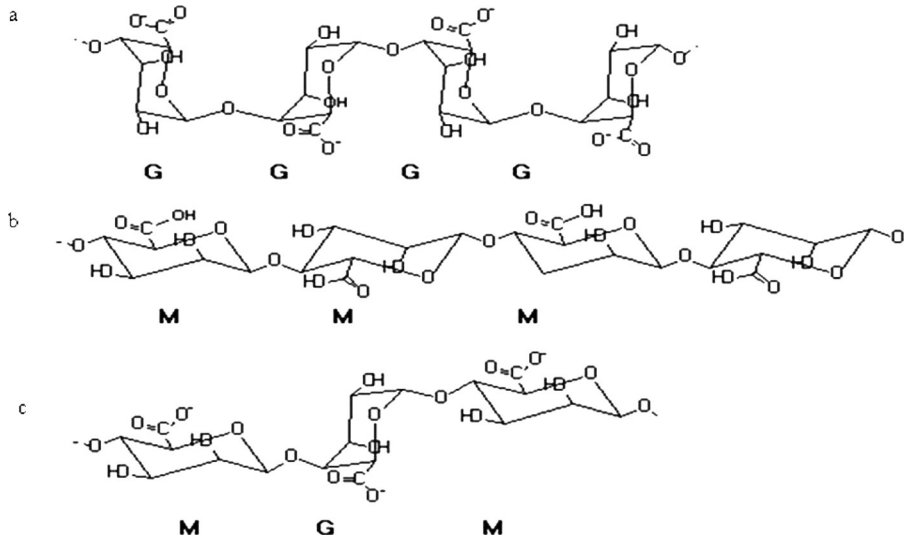

Figure 1. Chemical structures of alginate characterized by block sequences of (a) GG, (b) MM, and (c) MG.

yet to consider from the viewpoint of uronic acid composition of alginate. On the note of sustained drug release and mucoadhesion of a dosage form are envisaged to be greater with an increase in polymer molecular weight,[5,22] this study aims to evaluate high molecular weight M-rich and G-rich alginates from the perspective of their capacity as an oral insulin nanoparticulate carrier. *In vitro* drug release and *in vivo* antidiabetic characteristics of these nanoparticles are examined against those of low molecular weight alginates to identify functional oral insulin carrier and their structural influences on blood glucose lowering attribute.

MATERIALS AND METHODS

Materials

Four grades of M- and G-rich sodium alginates (ISP, Waterfield, Tadworth) were employed as the matrix polymer of nanoparticles (Table 1) with calcium chloride dihydrate (Fluka, Prague, Czech Republic) as cross-linking agent and bovine insulin (Sigma–Aldrich, St. Louis, Missouri) as drug. Sodium hydroxide and hydrochloric acid (Merck, Darmstadt, Germany) were chemicals used in pH adjustment of processing materials. Ultrafiltration membrane (polyethersulfone; Millipore Corporation, Billerica, Massachusetts) with nominal molecular weight limits of 100 and 10 kDa was used to harvest high and low molecular weight alginate nanoparticles respectively. Sodium hydroxide and potassium dihydrogen phosphate (Merck) were employed in preparation of phosphate buffer pH 6.8 USP for use as dissolution medium. Acetonitrile (Merck), trifluoroacetic acid (BDH, Poole, England), and ultrapure water processed at 18 MΩ were utilized to prepare the mobile phase for high-performance liquid chromatography (HPLC) analysis. Streptozotocin (Sigma–Aldrich), citric acid monohydrate (Merck), tri-sodium citrate (Fisher Scientific, Loughborough, Leicestershire, UK), and glucose (IDS Manufacturing Sdn Bhd, Shah Alam, Selangor, Malaysia) were chemicals employed in diabetic induction of rats. Other materials used in *in vivo* experiments included sodium chloride, methanol, and diethyl ether (Merck).

Formulation Variables of Nanoparticles

Insulin nanoparticles were prepared through ionotropic gelation of alginate solution with calcium chloride. The influences of alginate concentration, calcium chloride concentration, and pH of processing liquid on particle size, zeta potential, insulin association efficiency, and content of matrix were examined where applicable. High molecular weight G-rich alginate was used as the model polymer to streamline the working range of formulation variables. It was envisaged that formulation conditions applicable to viscous sample would be appropriate for other alginate grades (Table 1). The formulation conditions that produced particles over a size range encompassing nano- and micro-dimensions were selected.

Alginate and Calcium Chloride Concentration

Twenty gram of aqueous solution containing 0.025%, 0.050%, or 0.100% (w/w) alginate had its solution pH adjusted to 4. It was then added dropwise using a microsyringe (BD Ultra-Fine™ Needle, Becton, Dickinson and Company, Franklin Lakes, New Jersey) with a flow rate of 0.194 ± 0.021 g/s into 50 g of 0.003%, 0.006%, 0.012%, 0.025%, or 0.050% (w/w) calcium chloride solution under high-speed magnetic stirring at 1000 rpm and 25°C, similar to the methods adopted by Racovita et al.[23] The stirring was continued for an additional period of 15 min after the last addition of alginate solution. The dispersion was then subjected to particle size and zeta potential analysis. Triplicates were conducted and results averaged.

pH of Processing Liquid

An accurately weighed amount of insulin (3 mg) was dissolved in 20 g 0.0050 M hydrochloric acid solution. The insulin solution was then added dropwise into 90 g of alginate solution at pH 4 under high-speed magnetic stirring at 1000 rpm and 25°C to provide an aqueous mixture carrying 20 mg alginate and 3 mg insulin. A diluted alginate solution (90 g instead of 20 g liquid) was used to prepare alginate–insulin aqueous mixture. The reason was the large molecular weight alginates exhibited a high-interaction propensity with insulin solution. A simple mixing of 0.100% (w/w) alginate and 0.015% (w/w) insulin in