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Research paper

Natural dendrimers: Synthesis and in vitro characterization of glycogen-cysteamine conjugates

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ABSTRACT

The aim of this study was to synthesize, characterize and evaluate the mucoadhesive properties of the first thiolated hyperbranched natural polysaccharide with biodegradability and biocompatibility features. In detail, glycogen-cysteamine conjugates were synthesized through a first step of oxidative ring opening applying increasing concentrations of sodium periodate, to obtain polymers with different degrees of oxidation, and a second step of reductive amination with a constant amount of cysteamine. The obtained glycogen-cysteamine conjugates were characterized regarding their content of free and total thiol groups by Ellman's assay, biocompatibility, swelling/erosion behavior, rheological synergism and mucoadhesive properties in comparison to the unmodified glycogen. The higher the concentration of periodate was, the higher was the content of total thiol groups being in the range of 255.7 ± 12–1194.5 ± 82 μmol/g, biocompatibility remained unaffected by these structural changes. On the contrary, the mucoadhesive properties, evaluated by tensile, rheological synergism and rotating cylinder studies, appear to be influenced by the thiol groups concentration on the glycogen. In particular the glycogen-cysteamine conjugate exhibiting the highest degree of thiolation showed a 79-fold increase in viscosity over a time period of 8 h, as well as, remained attached on freshly excised porcine mucosa 32-fold longer than the unmodified polymer. The higher was the amount of conjugated thiol groups, the higher was the water absorption capacity of glycogen-cysteamine tablets in Simulated Intestinal Fluid pH 6.8 (SIF). The introduction of thiol moieties on polymer changed the characteristics of the polysaccharide by improving mucoadhesion properties. Therefore, this work represents the first study describing thiolated natural dendrimers as potential platform useful to realize appropriate mucoadhesive nanocarrier systems suitable to prolong mucosal residence time.

1. Introduction

Mucoadhesion is defined as the capacity of synthetic or biological macromolecules to adhere to mucosal tissues. Since the early 1980s the interest for the mucoadhesion has inspired and received a significant degree of attention for the development of novel bioadhesive polymers for mucosal delivery [1,2], for its localization at a given target site, potential to prolong the residence time at the site of absorption and to retain the formulation in intimate contact with the absorption site, thereby improving drug bioavailability. Furthermore, the use of mucoadhesive polymers allows to reduce the frequency and the dose of drug to be administered, leading to an improved patient compliance. Accordingly, various natural and synthetic polymers have been explored as mucoadhesive excipients. Recently, the focus has shifted to a new type of mucoadhesive excipients with thiol groups, which are capable of forming covalent bonds with the mucus layer covering mucosal tissues and provide much higher adhesive properties than polymers generally considered to be mucoadhesive [3]. The interaction mechanism of these thiolated polymers, or the so-called thiomers, is due to an oxidation process, thiol/disulfide exchange reactions, between the reactive thiol groups of the polymer and

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cysteine-rich subdomains of mucus glycoproteins [4]. Thiolated polymers, then, show improved mucoadhesive properties, which provide a prolonged residence time of delivery systems on mucosal tissues, as well as cohesive properties and modulate disintegration time of thiomer tablets, water absorption, swelling and the rheological behavior of the polymer in physiological conditions [5]. In order to explore this new class of mucoadhesive polymers and for the continuous increasing request of new biodegradable and biocompatible polymers, the aim of this study was to synthesize and characterize a new thiolated polymer with these features, such as glycogen. In the recent years, numerous thiomers have been synthesized from natural polymer and evaluated for mucoadhesive properties as polysaccharides, hyaluronic acid [6], dextran [7], chitosan [8], and alginate [9]. However, some natural polymers, e.g. alginate, are biocompatible, but not biodegradable in human organism [10].

Traditionally, the thiolation of polymers have focused primarily on linear polymers. The thiomers known so far have limited thiol content and may require the use of high polymer content in the dosage form to obtain covalent reasonable adhesion [11]. So, in this study we have used natural dendrimers, that can be conjugated with a high content of thiols due to their polyvalent nature. Moreover, due to their spherical architecture drugs can be efficiently incorporated in these nanocarrier systems. The unique structural characteristics of dendritic and hyperbranched macromolecules, which have a number of chain ends and a high degree of branching, leads to different physical properties compared to conventional linear polymers. Dendrimers, studied in the last 10–15 years, are synthetic highly branched macromolecules, spherical particles, and were extensively evaluated as delivery systems for a variety of pharmaceutical applications. In particular, they have been studied as nanocarriers for chemotherapeutic agents or for bioactive materials and as solubilizing agents [11].

Natural glycogen, a hyperbranched polysaccharide based on D-glucose units connected by (1 → 4)-α-glycoside bonds with branches every 7–11 residues that are joined by (1 → 6)-α-glycoside bonds (Fig. 1) [12,13], was chosen in this study also for its biodegradability and biocompatibility features. The glycogen morphology has been described in terms of rosette-like α-particles, a natural dendrimer, with a mean diameter of 20–30 nm and molecular weight between 10^6 and 10^7 Da [14–16]. The literature on glycogen modification strategies is poor and it has never been explored as possible mucoadhesive excipient. The usual strategies for the insertion of chemical functionalities on the polysaccharide backbone imply the chemical derivatization [17]. Therefore, the modification methods used for other polysaccharides can be used also for glycogen [18,19]. In particular, among the functionalization reactions, oxidation with periodate offers peculiar opportunities. Indeed, periodate salts form the corresponding aldehydes in the presence of vicinal diols [20]. In the present work, after obtaining glycogen with different oxidation degrees, it was conjugated with cysteamine and characterized regarding its size and size distribution, zeta potential, swelling/erosion behavior, rheological synergy and mucoadhesive properties, and biocompatibility.

2. Materials and methods

2.1. Materials

Glycogen (Polglumyt® highly purified glycogen, molecular weight of the order of 106 Da) was a gift of Angelini Research Centre, Pomezia (RM), Italy. 2-(N-Morpholino)ethanesulfonic acid (MES hydrate), 2,4,6-trinitrobenzenesulfonic acid (TNBS), 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent), cysteamine, ethylene glycol, hydroxylamine hydrochloride, sodium periodate ($NaIO_4$), 2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS), methyl orange, sodium borohydride ($NaBH_4$), sodium cyanoborohydride ($NaBH_3CN$) and dialysis tubing cellulose membrane (molecular weight cut-off of 12 kDa) were all purchased from Sigma–Aldrich, Vienna, Austria.

2.2. Oxidation of glycogen

Glycogen was modified to aldehyde forms, glycogen-CHO with a slight change comparison with the procedure described by Ito et al. [21]. Briefly, in 500 mL Erlenmeyer flask covered with aluminium foil, 1.5 g of glycogen were hydrated in 100 mL of water and increasing amounts (0.05; 0.150; 0.200; 0.250; 0.300 g) of $NaIO_4$, solubilized in 10 ml of water, were added to solution and stirred for 2 h at room temperature. The reactions were stopped by addition of 400 μL of ethylene glycol, needful for the inactivation of the unreacted periodate and then stirred for 1 h at room temperature. The resulting oxidized glycogen polymers were dialyzed exhaustively against water for 3 days. The water was replaced three times today. Afterwards, the purified polymers were lyophilized at −30 °C and 0.01 mbar (Christ Beta 1–8 K; Osterode am Harz, Germany) and stored at 4 °C until further use.

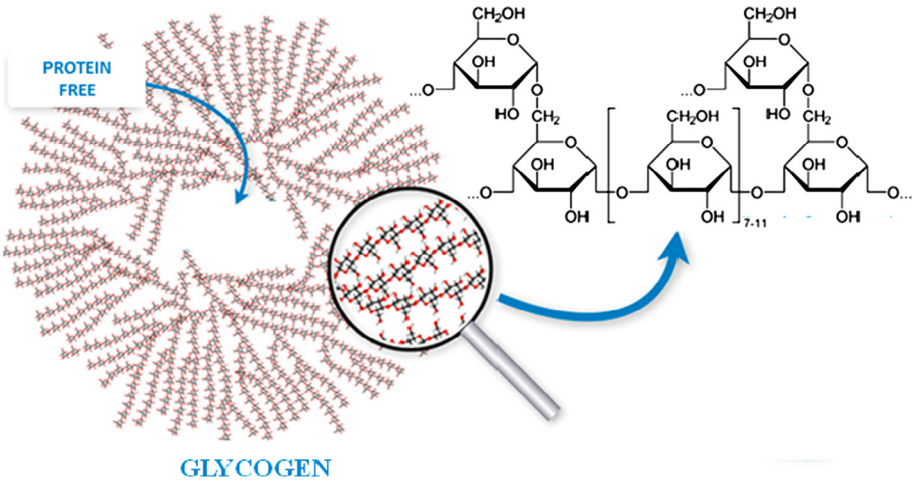

Fig. 1. Schematic diagram of highly purified natural glycogen. The figure shows the substructure of a hyperbranched polysaccharide based on D-glucose units connected by (1 → 4)-α -glycoside bonds with branches every 7–11 residues that are joined by (1 → 6)-α-glycoside bonds.