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Self-assembled carrageenan/protamine polyelectrolyte nanoplexes—Investigation of critical parameters governing their formation and characteristics

Maria Dul[a], Krzysztof J. Paluch[a,b], Hazel Kelly[a], Anne Marie Healy[a], Astrid Sasse[a], Lidia Tajber[a,*]

[a] School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland
[b] Bradford School of Pharmacy, School of Life Sciences, Centre for Pharmaceutical Engineering Science, University of Bradford, Norcroft Building, Richmond Road, Bradford BD7 1DP, W. Yorks., UK

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ABSTRACT

The aim of this work was to investigate the feasibility of cross-linker free polyelectrolyte complex formation at the nanoscale between carrageenan (CAR) and protamine (PROT). The properties of CAR/PROT nanoparticles (NPs) were dependent on the carrageenan type: kappa (KC), iota (IC) and lambda (LC), concentration of components, addition of divalent cations, weight mixing ratio (WMR) of constituents and mode of component addition. In the case of 0.1% w/v solutions, IC-based NPs had the smallest particle sizes (100–150 nm) and low polydispersity indices (0.1–0.4). A decrease in the solution concentration from 0.1% to 0.05% w/v enabled the formation of KC/PROT NPs. All carrageenans exhibited the ability to form NPs with surface charge ranging from −190 to 40 mV. The inclusion of divalent cations caused an increase in the particle size and zeta potential. Infrared analysis confirmed the presence of a complex between CAR and PROT and showed that IC chains undergo structural changes when forming NPs. Colloidal stability of NPs was related to the initial surface charge of particles and was time- and pH-dependent. IC was found to be the most suitable type of CAR when forming nanoplexes with PROT.

1. Introduction

Recent advances in pharmaceutical nanotechnology are focused on using polymeric nanoparticulate systems as carriers for drugs (Delie & Blanco-Prieto, 2005). Nanoparticles (NPs) formulated from natural polymers have attracted considerable interest (Hans & Lowman, 2002). NPs have many advantages, such as the potential to retain protein stability, increase duration of therapeutic effects of proteins and they can be administered by nonparenteral routes (Sarmento, Bibeiro, Veiga, Ferreira, & Neufeld, 2007). Natural polymers extensively studied for drug delivery purposes include polysaccharides such as alginate, chitosan, carrageenan (CAR) and proteins, for example casein and gelatin (Sonia & Sharma, 2012).

Polymers, especially those of natural origin, are often composed of subunits capable of bearing charge, thus the polyelectrolyte complexation method of NP preparation has received increasing attention in recent years. NPs formed by this method have several characteristic advantages for cellular uptake and colloidal stability, including suitable diameter, surface charge, spherical morphologies and low polydispersity indices (Bayat et al., 2008). Furthermore, the preparation of NPs by polyelectrolyte complexation methods can be carried out in completely aqueous conditions and at ambient temperature; therefore the stability and biological activity of loaded peptides would not be affected (Hu, Yang, & Hu, 2012; Ryan et al., 2013; Umerska et al., 2012, 2014).

Carrageenans (CARs) are an example of such polymers and are capable of forming polyanions. They are a family of hydrophilic sulphated biopolysaccharides extracted from various species of the *Rhodophyta* class of red seaweeds (Campo, Kawano, Braz da Silva, & Carvalho, 2009). CARs are composed of a hydrophilic linear sulphated backbone of alternating 1→3 glycosidic-linked β-D-galactopyransose units and 1→4 glycosidic-linked α-D-galactopyranose units or 1→4 glycosidic-linked 3, 6-anhydro-α-D-galactopyranose units (Fig. 1) (Berth, Vukovic, & Lechner, 2008) and principally differ in the number and position of the sulphate groups, however these reflect only general differences

* Corresponding author. Tel.: +00353 1 896 2787; fax: +00353 1 896 2810.
E-mail addresses: mdul@tcd.ie (M. Dul), K.J.Paluch@bradford.ac.uk (K.J. Paluch), hakelly@tcd.ie (H. Kelly), healyam@tcd.ie (A.M. Healy), sassea@tcd.ie (A. Sasse), lidia.tajber@tcd.ie, ltajber@tcd.ie (L. Tajber).

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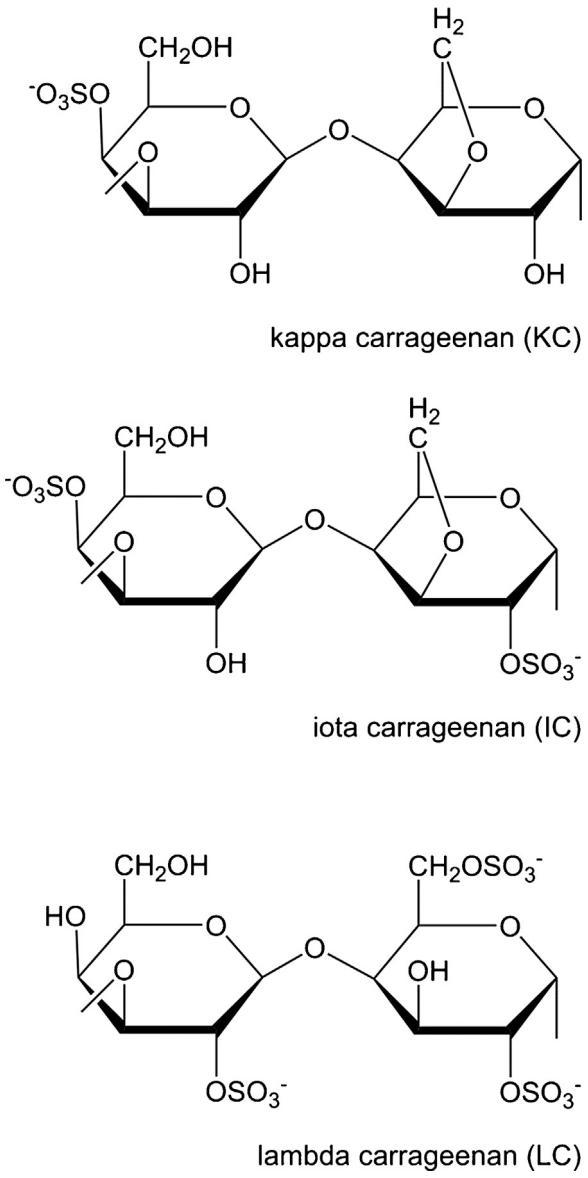

Fig. 1. Chemical structure of kappa carrageenan (KC), iota carrageenan (IC) and lambda carrageenan (LC).

in the composition and degree of sulphation (Necas & Bartosikova, 2013). The actual content of the sulphate residue (by weight) may vary between 15 and 40% for the various carrageenan types (Nanaki, Karavas, Kalantzi, & Bikiaris, 2010). Their applications include experimental medicine, pharmaceutical formulations and are well established as gelling, stabilising and thickening agents for food, cosmetics and industrial uses (Necas & Bartosikova, 2013). CARs are biocompatible and biodegradable polysaccharides with low toxicity and well documented properties of controlling and extending release of various drug substances (Nanaki et al., 2010) as well as improving apparent solubility and dissolution rates of poorly soluble actives (Dai, Dong, & Song, 2007). In addition, studies have shown that CARs have a high capacity to interact with proteins due to their strong ionic nature (Malafaya, Silva, & Reis, 2007) and form excellent matrices for predictable synthesis of magnetite nanoparticles (Daniel-da-Silva et al., 2007).

To form polyelectrolyte complexes (PECs) with CAR, protamine (PROT) was selected due to its reported *in vitro* membrane-translocating ability, believed to be associated with its positively charged polyarginine chains (Reynolds, Weissleder, & Josephson, 2005). The use of cell-penetrating peptides for drug delivery has been extensively studied over the past two decades (Temsamani & Vidal, 2004).

Shumilina and Shchipunov (2002) investigated interactions between CAR and chitosan (CS). They showed that the nature or type of CAR considerably influenced the characteristics of the PECs. The mechanical strength of PEC gels were ranked as follows: lambda carrageenan (LC)/CS > iota carrageenan (IC)/CS > kappa carrageenan (KC)/CS. Moreover, the gels obtained with IC and KC were temperature sensitive due to the helix-coil conformational transitions in their molecules (Shumilina & Shchipunov, 2002). An investigation was performed on the potential of PECs formed between KC, IC or LC and CS to form controlled release systems for glucose oxidase (Briones & Sato, 2010). The complex between CS and KC showed high encapsulation efficiencies for glucose oxidase while having the lowest release rate for this compound. Furthermore, this complex was able to protect the encapsulated glucose oxidase against degradation in pH 1.2 solution, in a chitosanase solution and in a pepsin solution (Briones & Sato, 2010). Another example of CS/CAR PECs used for delivery of proteins has been reported (Li, Hein, & Wang, 2013). As characterised by Li et al. (2013), in acidic solution the negatively charged sulphate groups of KC bound to positively charged amino groups of CS and formed acid-base PECs. When the pH was increased, amino groups protonated and the binding activity between both components became weaker, which resulted in swelling and disintegration of PEC and finally release of the protein. Modulation of drug release to the target site was possible by adjusting the factors that cause swelling properties of PEC (Li et al., 2013).

A number of publications show that carrageenans complex to CS (Briones & Sato, 2010; Li et al., 2013; Shumilina & Shchipunov, 2002), however no work has been published to date which indicates whether complexation between CAR and PROT is possible. Previous work by Umerska et al. (2014) showed that PROT/hyaluronate PECs can form, at the nanoscale, but depending on the ratio of constituents in such PECs their colloidal stability varies considerably. Therefore in this study we have focused on CAR/PROT systems with the aim of undertaking a methodical assessment of the various types of CAR, their concentration, weight mixing ratios, mode of preparation and the additional small cation addition during PECs formation to investigate if CAR/PROT nanoplexes can be obtained and to determine the characteristics of any such nanoplexes formed. CAR/PROT nanoplexes have the potential of being biocompatible, safe as well as capable of peptide binding and protection, as demonstrated for hyaluronate/PROT systems (Umerska et al., 2014), however key parameters determining their pharmaceutical suitability (such as optimum conditions of formation and stability) first need to be assessed.

2. Materials and methods

2.1. Materials

Iota carrageenan (IC, cat. no. C4014), kappa carrageenan (KC, cat. no. 22048), lambda carrageenan (LC, cat. no. 22049) and protamine (PROT) (as a sulphate salt, from salmon, cat. no. P4020) were obtained from Sigma-Aldrich. All other reagents and chemicals used were of analytical grade.

2.2. Preparation and characterisation of polymer and PROT solutions

Loss on drying for polymers was determined by thermogravimetry. A Mettler TG 50 module linked to a Mettler MT5