Characterization of α-carrageenan solution behavior by field-flow fractionation and multiangle light scattering

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Abstract

The salt mediated molecular conformation change of alpha (α)-carrageenan was studied in 0.1 M solutions of NaCl, NaI, and KCl. Asymmetric Field-Flow Fractionation with multiangle laser light scattering (AFFF/MALLS) detection was used to determine the average molecular weight, radius of gyration, and hydrodynamic radius which were in turn used to calculate the molecular density. In the presence of 0.1 M NaCl, an inert salt that does not promote gelation, α-carrageenan has a denser structure compared to κ-carrageenan of a similar molecular weight. A distinct and dramatic increase in the molecular weight (factor of 2) was observed for α-carrageenan in 0.1 M NaI compared to 0.1 M NaCl. This combined with only a slight change in the radius of gyration, suggests intermolecular interaction to a more compact structure (e.g., coaxial helices). A similar increase in molecular weight is observed in 0.1 M KCl, accompanied with an approximate 50% increase in the radius of gyration as well as an increase in polydispersity. This may also be attributed to intermolecular interaction with helix formation (coaxial or lateral) or may be due to K^+ cations interacting with naturally occurring residual ι-carrageenan in the sample. As previously reported for other carrageenans the random coil to helix transition of α-carrageenan appears to be stabilized by K^+ cation or I^- anion in an aqueous environment.
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1. Introduction

Carrageenans are linear sulfated galactans obtained from red seaweeds (Rhodophyceae) that are commonly used as thickeners, gelling agents, and stabilizers in the dairy and cosmetic industries. These polysaccharides may contain up to 1000 galactose residues with various sulfate ester substitutions and as a result many structures are possible. The structures are classified into three main commercial types based on the location of the sulfate esters and the presence or absence of the 3,6-anhydro ring (Fig. 1): kappa (κ), iota (ι), and lambda (λ) with the different types exhibiting different gelling properties due to salt mediated dimerization and/or temperature (Marcelo, Saiz, & Pilar Tarazona, 2005; Piculell, 2006).

The gelation mechanism of κ- and ι-carrageenans has been previously described and investigated (Cuppo, Reynaers, & Paoletti, 2002; Mangione, Giacomazza, Bulone, Martorana, & San Biagio, 2003; Viebke, Borgström, Carlsson, Piculell, & Williams, 1998; Yuguchi, Thành, Urakawa, & Kajiwara, 2002); λ-carrageenan appears not to gel in aqueous systems. Briefly, a random coil to helix conformational transition proceeds with aggregation of helical segments to rigid rod bundles, eventually forming a gel network. Although there is a controversy concerning the exact intermolecular helix interaction, Piculell (2006) has proposed that most reported data support a coaxial double helix conformation as opposed to lateral interaction.

Both anions and cations contribute to molecular processes associated with conformational transitions and gelation of carrageenans. For instance κ-carrageenan forms a strong gel in the presence of potassium, rubidium, or cesium ions and a weaker gel with calcium ions;

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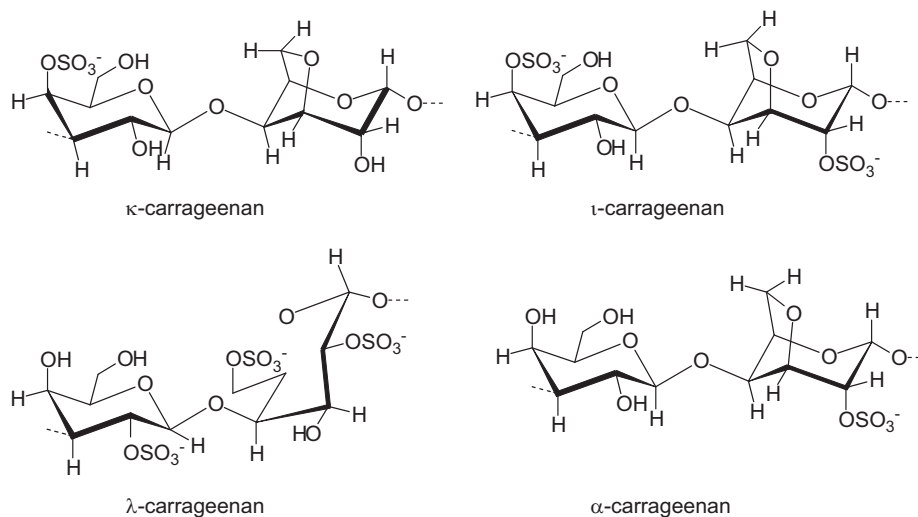E-mail address: janet.poli@sanofipasteur.com (J.B. Poli).

Fig. 1. Chemical structure of ideal disaccharide repeat units for some carrageenans.

ι-carrageenan with alkali metal cations forms weak gels, while with alkali earths it forms transparent and rigid gels (Yuguchi, Urakawa, & Kajiwara, 2003). Cations tend to stabilize helix formation, whereas anions seem to have no influence, except in the case of iodide. It has been proposed that for κ-carrageenan, iodide ions stabilize helices through binding in interior cavities but at the same time may prevent aggregation and gelation by creating a relatively hydrophobic microenvironment (Grasdalen & Smidsröd, 1981; Nerdal, Haugen, Knutsen, & Grasdalen, 1993).

Alpha (α)-carrageenan was first reported by Zablackis and Santos (1986) and was further characterized by Falshaw et al. (1996). Studies by Nuclear Magnetic Resonance (NMR; Falshaw et al., 1996), infrared spectroscopy (IR; Zablackis and Santos, 1986) and Gas Chromatography with Mass Spectrometry detection (GC/MS; Chiovitti et al., 1997) confirm that α-carrageenan is composed of alternating three linked β-D-galactopyranosyl and four linked 3,6-anhydro-α-D-galactopyranosyl-2-sulfate units. Zablackis and Santos (1986) reported that the sodium form of α-carrageenan did not gel, yet exhibited high viscosity, and exhibited twice the capacity of κ-carrageenan to suspend cocoa particles in milk.

The solution behavior (e.g., varying temperature or ionic strength conditions) of many carrageenans has previously been studied. Techniques used include size exclusion chromatography with multiangle light scattering detection (SEC-MALS; Marcelo et al., 2005), single angle X-ray scattering (SAXS; Thành et al., 2002; Yuguchi et al., 2002, 2003), NMR (Falshaw et al., 1996; Grasdalen & Smidsröd, 1981; Nerdal et al., 1993), and asymmetric field-flow fractionation (AFFF; Viebke & Williams, 2000; Wittgren, Borgström, Piculell, & Wahlund, 1998).

AFFF has the advantage of fractionating large macromolecules while avoiding the problem of polymer particles strongly absorbed on packing material or being totally excluded in an SEC column. In practice the AFFF covers a size range from 5 nm up to approximately 100 μm and molecular weights (Mw) from 10 to 10^{12} kDa, which is much higher than that of SEC. Thành et al. (2002) determined the average Mw range for different carrageenans between 147 and 2399 kDa and a size range of 60.7–109 nm. It is anticipated that α-carrageenan will behave in a similar manner with molecular characteristics in this range, thus AFFF is used in this investigation for molecular characterization in solution.

A comprehensive description of macromolecular structure includes molecular shape as well as coil density and flexibility. The hydrodynamic radius R_h is calculated from the retention by AFFF while simultaneous MALS detection measures the radius of gyration R_g and molar mass subsequent to separation. The mean coil density is then calculated as the molecular density ρ which is the ratio of R_g/R_h. Theoretical values for molecular densities which correspond to various polymer shapes are: $\rho = 0.775$ for compact sphere, $\rho = 1.862$ for flexible polymer in a good solvent, and $\rho = 2.659$ for a rigid rod with an axial ratio of 100 (Wittgren et al., 1998).

1.1. Asymmetric field flow fractionation

AFFF has previously been described by many (Wahlund, 2000) and is briefly summarized here. AFFF is a chromatography-like technique where the separation occurs in a thin channel, usually 100–500 μm thick. A sample is injected into the channel and focused onto the accumulation wall. Next the sample components are allowed to diffuse by Brownian motion into the channel. Each sample component obtains a characteristic steady-state equilibrium distance from the wall depending on its diffusion coefficient. A flow through the channel is initiated and the elution order based on diffusion coefficient is obtained. The separation is further optimized with the application of a constant or variable secondary crossflow to accomplish desired resolution. Subsequent to separation, molar mass and R_g is determined from the light