Gelation of carrageenan: Effects of sugars and polyols

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A B S T R A C T

The gelation of κ-carrageenan, a common polysaccharide for food gelling and thickening, is enhanced by the addition of sugars and polyols. Three different hypotheses have been proposed to explain this phenomenon: (i) the enhancement of the water structure around polysaccharides, and the concurrent change in polysaccharide hydration, induced by the cosolvent; (ii) exclusion of cosolvents from polysaccharide surfaces; (iii) binding between sugars/polyols and polysaccharides in the gel phase. To examine the above hypotheses, as well as to reveal the true driving forces responsible for gelation enhancement, we applied our recent statistical thermodynamic theory of gelation, derived from the rigorous Kirkwood—Buff theory. The hydration change is shown to be negligibly small relative to cosolvent exclusion and cosolvent-biopolymer binding, which can be rationalized by considering a sol—gel equilibrium in which (1) the exclusion of sugars/polyols from κ-carrageenan's surface in the sol phase, and (2) the binding of sugars and polyols in the gel phase, shift the sol—gel equilibrium of κ-carrageenan to the gel state. This novel picture is consistent with a wealth of experimental evidence and provides a mechanistic insight into how polyol and sugar cosolvents influence the gelation of κ-carrageenan on a molecular level.

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1. Introduction

Carrageenan, an assortment of sulphated polyelectrolyte heteropolysaccharides extracted from the red seaweed, has long been used in food (Burey, Bhandari, Howes, & Gidley, 2008; Necas & Bartosikova, 2013; Prajapati, Maheriya, Jani, & Solanki, 2014; Saha & Bhattacharya, 2010; Saltmarsh, 2015). One member of the carrageenan family, κ-carrageenan (Fig. 1), has been employed ubiquitously as a food additive because of its thickening and gelling capabilities (Burey et al., 2008; Necas & Bartosikova, 2013; Prajapati et al., 2014; Saha & Bhattacharya, 2010; Saltmarsh, 2015). The gelation of κ-carrageenan, like other polysaccharides, has been known to be strongly affected by cosolvents, in particular such as sugars and polyols; such cosolvents have therefore been exploited widely to enhance κ-carrageenan gelation and gel stability (Arakawa & Timasheff, 1982; Gekko, Mugishima, & Koga, 1987; Loret, Ribelles, & Lundin, 2009; Nishinari & Watase, 1992; Nishinari, Watase, Williams, & Philips, 1990; Oakenfull, 2000;

Ramakrishnan & Prud'homme, 2000). What sugar/polyol cosolvents do to the gelation of κ-carrageenan is well known; however, how these cosolvents actually enhance gelation, *at a molecular level*, remains a mystery, and has been a subject of intense debate for decades (Arakawa & Timasheff, 1982; Gekko et al., 1987; Loret et al., 2009; Nishinari & Watase, 1992; Nishinari et al., 1990; Oakenfull, 2000; Ramakrishnan & Prud'homme, 2000). Hence the aim of this paper is to provide a clear explanation, based upon a statistical thermodynamic foundation, into how exactly sugar and polyol cosolvents influence the gelation of biopolymers by examining the gelation of κ-carrageenan.

A number of hypotheses have been proposed regarding how cosolvents affect the gelation of biopolymers; these hypotheses can be classified into the following three categories: (i) cosolvent-induced changes in the water structure, and the consequent hydration change of the biopolymer, induced by the cosolvent (Arakawa & Timasheff, 1982; Back, Oakenfull, & Smith, 1979; Gekko et al., 1987; Nishinari & Watase, 1992; Oakenfull, 2000); (ii) preferential exclusion of the cosolvent from the biopolymer surface (Back et al., 1979; Shimizu & Boon, 2004; Shimizu & Matubayasi, 2014a, b; Timasheff, 2002a, b) and (iii) direct binding of the cosolvent to the biopolymer chain in the gel state (Kumar, Modig, &

Abbreviations: KB, Kirkwood—Buff.
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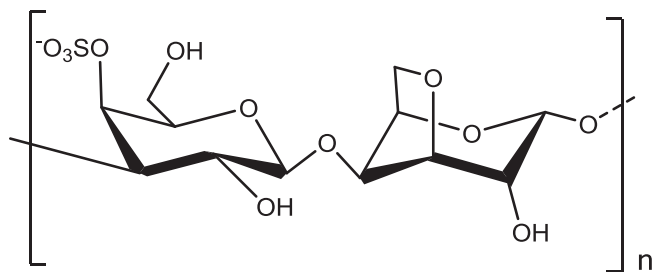*E-mail address:* seishi.shimizu@york.ac.uk (S. Shimizu).

Fig. 1. Chemical structure of κ-carrageenan.

Halle, 2003; Loret et al., 2009; Makhatadze & Privalov, 1992; Prakash, Loucheux, Scheufele, Gorbunoff, & Timasheff, 1981; Ramakrishnan & Prud'homme, 2000). We will begin by briefly examining each hypothesis.

1.1. Cosolvent-induced change of water structure and hydration change

This hypothesis stems originally from the classical view of biomolecular folding and stability, in which the hydrophobic effect has long been believed to play a dominant role (Dill, 1990; Frank & Evans, 1945; Kauzmann, 1959; Tanford, 1968). According to this view, the hydrophobic effect is caused by the entropy penalty due to the enhancement of hydrogen bonds between water molecules adjacent to the hydrophobic solute (Dill, 1990; Frank & Evans, 1945; Kauzmann, 1959; Tanford, 1968). Hence cosolvent molecules that enhance the hydrogen bond network of the surrounding water molecules, known formally as kosmotropes, are considered to concurrently enhance the hydrogen bond network around the hydrophobic solutes, thereby strengthening the hydrophobic effect and, consequently, facilitating biomolecule–biomolecule association (Dill, 1990; Frank & Evans, 1945; Kauzmann, 1959; Tanford, 1968).

The enhancement of the water structure by the cosolvent is thus the cause of the hydration change. Attempts have therefore been made to measure, or obtain evidence for, the cosolvent-induced changes in hydration accompanying gelation (Dill, 1990; Frank & Evans, 1945; Gekko et al., 1987; Kauzmann, 1959; Oakenfull, 2000; Tanford, 1968; Uedaira, Ikura, & Uedaira, 1989). A bulk of evidence has been reported to support this view: (i) the introduction of cosolvents changes the gelation process from an enthalpically driven to an entropically driven process, indicating a cosolvent-induced release of water (Gekko et al., 1987); (ii) the increase in the isothermal compressibility of κ-carrageenan gels, indicative of water release (Gekko & Kasuya, 1985); (iii) the observed linear correlation between the "water structure parameter" (derived from the partial molar volume of the cosolvent) and the cosolvent-induced increase in thermal stability of the gel (Oakenfull, 2000); this is underscored further by a linear correlation between the "water structure parameter" and the number of equatorial-OH groups on the cosolvent (Nishinari & Watase, 1992; Oakenfull, 2000; Uedaira et al., 1989) which correlates also with the sugar-enhanced thermal stability of κ-carrageenan gels (Nishinari & Watase, 1992).

In contrast to all above, our recent statistical thermodynamic study on the gelation of agarose and gelatin has shown that the contribution of cosolvent-induced hydration change is negligible (Shimizu & Matubayasi, 2014b). However, the question still remains as to whether this is also the case for κ-carrageenan gelation. Also the previous evidence for this hypothesis, as summarised above, should be examined critically in the light of statistical thermodynamics.

1.2. Preferential exclusion of cosolvents from polysaccharide chains

This hypothesis also originates from biomolecular hydration thermodynamics; the enhancement of biomolecular association in the presence of cosolvents can be rationalised by the preferential exclusion of cosolvents (or equivalently, preferential hydration) from the biomolecular surface (Timasheff, 2002a, b) which has been supported by experiments (Casassa & Eisenberg, 1964; Tanford, 1970; Timasheff, 1998).

The notion of preferential cosolvent exclusion has sometimes been invoked to rationalise the cosolvent-induced enhancement of biopolymer properties (Back et al., 1979; Shimizu & Matubayasi, 2014a; Timasheff, 2002a). Shimizu and Matubayasi have recently extended the rigorous statistical thermodynamic theory of the cosolvent effect to gelation equilibria (Shimizu & Matubayasi, 2014b), which afforded a rationalisation for the sugar- and polyol-induced enhancement of gelatin and agarose gelation in terms of cosolvent preferential exclusion. What has emerged from this rigorous statistical thermodynamic theory is the clarification that preferential exclusion is actually caused not by the increased hydration but by the exclusion of cosolvents from biomolecular surfaces (Shimizu & Matubayasi, 2014b). Hence this hypothesis is actually about the exclusion of cosolvents. Whether this is the case also for κ-carrageenan remains to be examined.

1.3. Binding of cosolvents on polysaccharide gels

Sugars and polyols bind directly to κ-carrageenan, increasing the number of junction zones, with shorter average length, per unit volume (Nishinari & Watase, 1992; Oakenfull, 2000). This is attributed to the stabilization of the characteristic intermolecular hydrogen bonding between individual κ-carrageenan strands in a typical junction zone by the formation of intermolecular, cross-linking hydrogen bonds between the OH-groups of the sugar/polyol cosolvent and κ-carrageenan (Nishinari & Watase, 1992; Oakenfull, 2000).

Such a change in cross-linking came originally from rheological evidence (Loret et al., 2009; Nishinari & Watase, 1987), as well as the cosolvent-induced elevation of the gel denaturation temperature for agarose gels (Watase, Kohyama, & Nishinari, 1992) and κ-carrageenan gels (Gekko et al., 1987; Nishinari & Watase, 1992; Nishinari et al., 1990). Such evidence, more specifically, includes: i) the shorter average length, and therefore increased number per unit volume, of junction zones for κ-carrageenan gels in the presence of sugars/polyol cosolvents (Nishinari & Watase, 1992; Oakenfull, 2000); ii) the decreased free energy of junction zone formation; due to stabilisation of intermolecular cross-linking of individual κ-carrageenan molecules in the junction zones by intermolecular hydrogen-bonding between cosolvent OH-groups and individual κ-carrageenan strands (Oakenfull, 2000); iii) the increased setting and melting temperature of κ-carrageenan gels in the presence of sugars (Nishinari & Watase, 1992; Nishinari et al., 1990; Ramakrishnan & Prud'homme, 2000); iv) the increased rigidity of κ-carrageenan gels with increasing sugar/polyol concentration (Loret et al., 2009); v) the change in rheological properties of κ-carrageenan gels above a critical sugar concentration (Loret et al., 2009); and vi) the positive correlation between the number of equatorial-OH groups on a sugar and the increased thermal stability of κ-carrageenan gels as equatorial groups are suspect to form cross-linking hydrogen bonds between biopolymers (Nishinari & Watase, 1992; Oakenfull, 2000).

Similar observation and explanations have been reported for