Structure-Function Relationships in Carrageenans and Agarose

Dr. Srinivas Janaswamy

Whistler Center for Carbohydrate Research

Purdue University
Polysaccharides

- Flavor Encapsulation
- Crystallization Inhibition
- Coating ability
- Hygroscopicity
- Sweetening Effect
- Cryoprotection
- Solubility
Polysaccharides

- Anti-Coagulant
- Anti-Therapeutic
- Anti-Tumor
- Reduction in Blood Cholesterol and Lipid levels
- Anti-HIV
Polysaccharides systematic study — Source → Molecular structure → Assembly → Solution Properties — provides better insights about their structure-function relationships and guides in their improved utility in food and non-food applications. Further, knowledge about their interactions with protein/virus molecules helps in the development of polysaccharide-based vaccines.
Why do we Need the Knowledge about Polysaccharides Structure-Function Relationships?

• Polysaccharides have extensive group of different chemical structures and exhibit a wide variety of unique molecular structures leading to sheets and spirals of single, double and triple helices.

• The structural forms, obtained in the solid state, appear to remain unaltered even in the hydrated environment of the polysaccharide solutions and gels.

• Hence, knowledge of polysaccharides three-dimensional structure, through fiber diffraction analysis, and rheological behavior of their solutions as well as their intrinsic relationships are very much necessary for their effective utilization in food and non-food applications.
Carrageenans
Seaweeds from Galveston’s Beaches

Green Algae

Brown Algae

Red Algae
Carrageenans

- Fifteen Carrageenans: κ, τ, λ, θ, μ, ν, ξ, α, β, ω, ο, π, γ, δ and ψ
- κ, τ, θ, α, β and ω contain 3, 6-anhydro-bridge in the α-D-galactose residue
- κ, τ, λ, μ and ν are commonly found in most of the seaweed specimens
Carrageenans

- κ-, τ- and λ-carrageenans are utilized extensively due to their greater versatility.
- They carry one, two and three sulfate groups, respectively, per disaccharide repeat.
- In the presence of mono and divalent cations, gelation occurs for κ- and τ-carrageenans, but λ-carrageenan does not gel at any concentration.
- Gels of κ-carrageenan are relatively hazy and brittle while τ-carrageenan develops very clear and elastic gels that are free from syneresis.

Food: Instant and cooked pudding, whipped cream, Yogurt, chocolate milk, soft drinks, fruit juice, beer and wine, bakery goods, candies, salad dressing, sauces and jellies.

Bathroom: Toothpaste, shampoo, air freshener, lotion and shaving cream.

Medicine: Cough medicine and laxatives.

Pet food: Canned dog food.

Other: Paper marbling.
\(-\text{Carrageenan}\)
Solution Properties of \( \iota \)-Carrageenan

Free standing gels by different cation forms of \( \iota \)-carrageenan.

Variation of elastic modulus of \( \iota \)-carrageenan with cation type.

Solution Properties of $\tau$-Carrageenan

- **Na$^+$ form**
- **Ca$^{2+}$ form**
- **Na$^+$ form + 50mM NaCl**

**Graphs:**
- $G'/G''$ (Pa) against Temperature (°C)
- **Na$^+$ form**
- **Ca$^{2+}$ form**
- **Na$^+$ form + 50mM NaCl**

- Graphs show the modulus ratio $G'/G''$ at various temperatures for different forms of carrageenan and a mixed form with 50mM NaCl.
X-ray Fiber Diffraction Patterns of \( \tau \)-carrageenan

\[ \text{Na}^+ \]

\[ \text{Ca}^{2+} \text{ (II)} \]
Molecular Structure

- Three-fold, right-handed, parallel, half-staggered, double helix of pitch 2c
Helix-Helix Association

Sodium

Calcium

Cation-Induced Polymorphism and Pseudo-polymorphism
Association in Two Polymorphs

Ca$^{2+}$ Polymorph II

Ca$^{2+}$ Polymorph III
Is Half-Staggered a Molecular Trait?

\[
\begin{array}{cccc}
  & \text{Zn}^{2+} & & \text{Ca}^{2+} \\
  \text{Ni}^{2+} & & & \\
  6 & 5 & 2 & 3 \\
  4 & 3 & 2 & 2 \\
  2 & 1 & 2 & 0 \\
  4 & & & 0 \\
  6 & & & \\
\end{array}
\]
What is the root cause?

• Across the (1→3)-linkage, the 4-S group is displaced by about -36° around the helix axis and 3.6 Å from the 2-S group; in contrast across the next (1→4)-linkage, the 2-S group is rotated by -156° and translated by 5.0 Å from the 4-S group.

• Thus, the virtual bonds connecting adjacent sulfur atoms across the linkages are 5.0 and 11.4 Å, respectively.

• The half-staggering relationship between the two chains brings the 2-S group of one chain to almost the same z-level as the 4-S group of the other so that the 4-S···2-S separation is 7.1 Å.
What is the root cause?

- This interdigitation further leads to a contiguous arrangement of the sulfate groups 2-S (chain 1), 4-S (chain 2), 2-S (chain 2) and 4-S (chain 1) along the double helix surface.

- This results in a left handed helix in which successive S⋯S distances alternate between 7.1 and 5.0 Å
What is the root cause?

Interactions with two or more guest molecules

2-S···Ion···W···2-S  4-S···W···W···4-S
4-S···Ion···W···W···W···4-S  2-
S···W···Ion···4-S
4-S···Ion···W···Ion···4-S
2-S···W···W···W···4-S
κ-Carrageenan
Solution Properties

Frequency dependence of 1.2% kappa-carrageenan with 0.1 mol/dm$^3$ salt concentration.

*Biomacromolecules 2, 946-951, 2001.*

Frequency dependence of kappa-carrageenan in 0.2 mol/dm$^3$ NaI (circles) and 0.2 mol/dm$^3$ KCl (squares).

Diffraction Patterns

ι-Carrageenan

<table>
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<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
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κ-Carrageenan

| 6 |
|---|---|---|---|

1. Trigonal

- $a = 23.6$ and $c = 13.2$ Å
- $a = 26.7$ and $c = 25.2$ Å
Tako’s Model

--- electrostatic force of attraction
... ionic bond

Bridge: $K^+$, $Rb^+$ & $Cs^+$  YES
$Li^+$ & $Na^+$  NO
Association of \(\iota\)-carrageenan helices
κ-carrageenan helices Association
Anti-Parallel Double Helix

O6A ⋅⋅ O6A
O2A ⋅⋅ O2A
O2B ⋅⋅ O2B

Non-half staggered Model
Packing
\( \lambda \)-Carrageenan
$\text{Na}^+ \lambda$-Carrageenan + FeCl$_3\cdot$6H$_2$O

$\text{Fe}^{3+}$-Lambda-Carrageenan

$\text{Na}^+\text{-Lambda-Carrageenan}$

![Graph showing temperature vs. moduli (G')](#)
\( \lambda \text{-Carrageenan} + \text{FeCl}_3 \)
Domain Model of Carrageenan Gelation

Random coils → Ordered "domains" → Aggregated "domains"