

Structural Principles of Polysaccharide Gels

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The author and colleagues have proposed a gelation mechanism for κ -carrageenan, ι -carrageenan, agarose (agar), gellan gum, curdlan, amylose, and alginic acid at the molecular level in aqueous solutions in preceding papers. The synergistic gelation mechanism between xanthan gum and galactomannans (locust bean, tara-bean and guar gum), and konjac glucomannan, where trisaccharide side-chains of the former molecule take part in the interaction, have been proposed. The synergistic gelation mechanism between κ -carrageenan and galactomannan (locust-bean gum) has also been proposed. Recently, we have proposed gelatinization and retrogradation mechanisms of rice starch. The polysaccharides in water changed into an ice-like structure with hydrogen bonding between polymer and water molecules, and between water molecules even at a concentration range of 0.1–1.0% (w/v) at room temperature, resulting in gelation. Such dramatic changes from liquid into gels have not yet generally been understood at the molecular level. The research conducted by the author now makes it possible to discuss, in principle, because structure-function relationships from the viewpoint of rheology not only of the gelling polysaccharides mentioned above, but also of non-gelling welan, rhamsan, S-657 gum, schizophyllan, and amylopectins (rice and potato), the structure of which is similar to that of gelling gellan gum, curdlan and amylose, have also been analyzed at the molecular level in our preceding papers.

In the course of the rheological studies of polysaccharides, we have discussed the molecular origin for their rheological characteristics and proposed a gelation mechanism for κ -carrageenan,^{1,2)} ι -carrageenan,³⁾ agarose (agar),⁴⁾ gellan gum,⁵⁻⁹⁾ curdlan,^{10,11)} amylose,¹²⁻¹⁴⁾ and alginic acid¹⁵⁾ at the molecular level in aqueous solutions. We also proposed synergistic gelation mechanism for a mixture solutions of xanthan gum and galactomannans (locust-bean, tara-bean, and guar gum),¹⁶⁻²⁰⁾ and konjac glucomannan.²¹⁻²³⁾ where trisaccharide side-chains of the former molecules take part in the interaction; the model of which might provide the existence for D-mannose-specific binding sites in the host-pathogen relationships and in the cell recognition processes.^{18,20,21)} Furthermore, the synergistic association site between κ -carrageenan and galactomannan (locust-bean gum) has also been proposed at the molecular level.²⁴⁾ This model may also provide the existence for D-mannose-specific

binding sites between cell-surface proteins and mannose-rich oligo- and polysaccharides. Recently, we have proposed a gelatinization mechanism of rice starch at the molecular level in aqueous solution.²⁵⁾ The short side-chains of rice amylopectin molecules might take part in intermolecular hydrogen bonding with amylose molecules. A mechanism involving short-side chains playing a dominant role in the gelatinization of rice starch has been suggested from the synergistic gelling model with xanthan gum and galactomannans, and glucomannan.

Consequently, we have realized that there are some basic rules in the gel-formation processes of the polysaccharides in aqueous solutions. The gelation might occur in the formation of intra- and intermolecular associations, where hemiacetal oxygen, hydroxyl or methyl groups of the sugar residues of the polysaccharides contribute, with hydrogen bonding or van der Waals forces of attraction

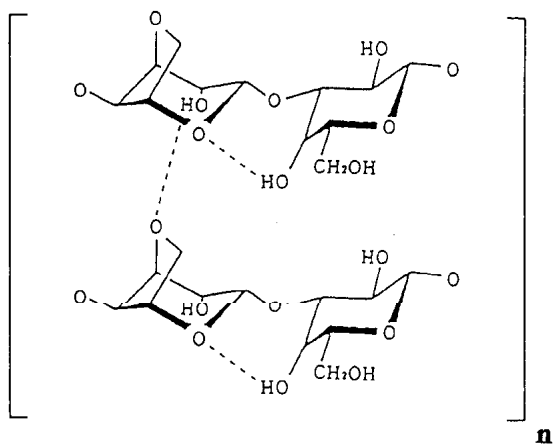
in aqueous solutions. The sulfuric acid and carboxyl groups of sugar residues of some acidic polysaccharides, such as κ -carrageenan, ι -carrageenan, gellan gum and alginic acid, might also participate in intra- or intermolecular associations through univalent or divalent cations with ionic bonding or electrostatic forces of attraction.

The gelling polysaccharides mentioned above, at a minimum concentration of 0.1 to 1.0% (w/v) in water (99.9–99.0%), changed into an ice-like structure with the formation of hydrogen bonding between the polysaccharides and water, and between water molecules, resulting in gelation at room temperature. Though increasing attention has been paid in recent years to the polysaccharide gels, such dramatic changes have not yet been generally understood at the molecular level. Thus, the research mentioned above has made it now possible to generalize.

We discuss herein the role of polysaccharides for an example agarose⁴⁾ in gel-formation processes in water. Agarose is the major constituent of agar obtained from a family of red seaweeds and used in the separation, purification and characterization of biomolecules in such techniques as gel filtration, affinity chromatography, hydrophobic chromatography and electrophoresis, as well as in the preparation of immuno adsorbants and immobilized enzymes.⁴⁾ It consists of a copolymer alternating *O*-3-linked β -D-galactopyranosyl and *O*-4-linked 3,6-anhydro- α -L-galactopyranosyl residues, the structure of which is similar to that of κ - and ι -carrageenan, except for the sulfate content and L-configuration. As illustrated in Scheme 1, we have proposed a gelation mechanism of agarose molecules in aqueous solution.⁴⁾ Intramolecular hydrogen bonding may take place between the OH-4 of the β -D galactopyranosyl residue and the adjacent hemiacetal oxygen atom of the anhydro- α -L-galactopyranosyl residue. This bonding is likely owing to the flexibility of the α -L-(1 \rightarrow 3) linkage between the anhydro-L-galactopyranosyl and D-galactopyranosyl residues. The anhydro- α -L-galactosyl residue is a cage-like sugar residue that contributes to the rigidity of the molecular chain of agarose, and stabilizes the proposed intramolecular hydrogen bonding even at high temperatures $>60^\circ\text{C}$.

Intermolecular hydrogen bonding may be involved in between the ring *O*-3,6 atom and OH-2, which has an axial orientation and is bound with less flexibility, of anhydro-L-galactopyranosyl residues on different molecules. This intermolecular hydrogen bonding may results from the cage effect,²⁶⁾ which may lead the lowest energy state of electrons of the lone pairs of ring oxygen atoms of the anhydro- α -L-galactosyl residues, the conformation of which may adopt a tetrahedral distribution, and therefore, may attract not only each other but also water molecules with hydrogen bonding. The cavity of the helix is occupied by water molecules, the arrangement of which is a nearly tetrahedral ice-like structure, in a manner similar to that of the anyhydro- α -L-galactopyranosyl residues. The intra- and intermolecular hydrogen bonding of the agarose molecules results in gelation up to a high temperature (60°C), and their periphery is surrounded by hydrophobic carbon and hydrogen atoms which may be required to play a role in the hydrophobic effect,^{27,28)} which may lead to hydrogen bonding easily within water molecules caused by a decrease in entropy. Therefore, hydrogen bonding may also take place easily through water molecules on the outside of the polymer helices. The molecules of agarose may adopt a random coil $>60^\circ\text{C}$, but may keep a rod-like conformation at the temperature range from 60 to 40°C owing to the formation of intramolecular hydrogen bonding. Intermolecular hydrogen-bonding may take place at temperatures $<40^\circ\text{C}$ even at a low concentration (0.1).⁴⁾

The H_2O molecule can participate in four hydrogen bonds (Fig. 1), two of them involving the two hydrogens of the molecule and the lone pair of electrons of the oxygen and hydrogens of two neighboring molecules.^{29–31)} This tetrahedrally directed bonding is involved in the crystal structure of ice (Fig. 2), which is the same as the arrangement of the gelling water molecules in agarose solution. The agarose molecules (0.1%), adopting hexagonal rigid conformation involving intra- and intermolecular hydrogen bonding (Scheme 1), has changed about 99.9% of the liquid water molecules into a network of tetrahedrally hydrogen-bonded water molecules (Fig. 2) even at room



Scheme 1. Possible model of intra- and intermolecular hydrogen bonding of agarose molecules in aqueous solution.

The dotted lines represent hydrogen bonding.

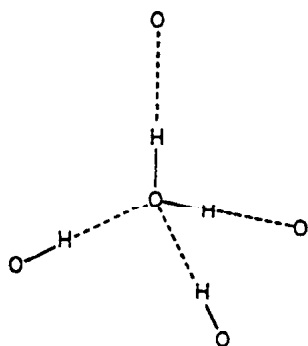


Fig. 1. Tetrahedral hydrogen bonding of water (H_2O) in ice. The dotted lines represent hydrogen bonding.

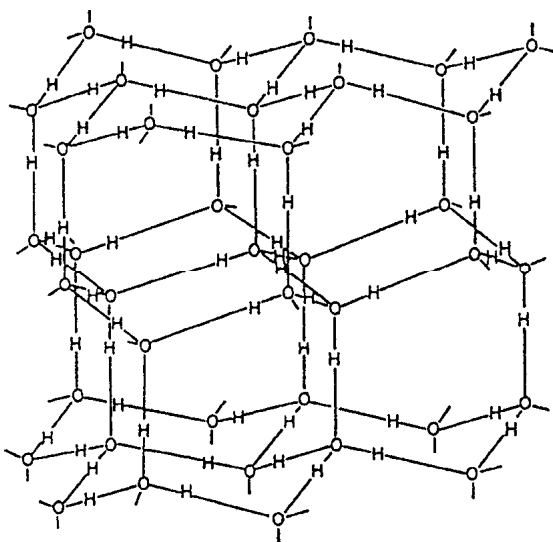


Fig. 2. Crystal structure of ice.

temperature, resulting in gelation. The ring oxygen ($>\text{O}$) and hydrocarbon (CH) groups of the agarose molecules may be liable for arranging a network of tetrahedrally hydrogen-bonded water molecules caused by cage²⁶⁾ and hydrophobic effect^{27,28)}, leading to the lowest energy state of the electrons of the lone pair of water molecules and extending into hydrogen bonding even at room temperature,³¹⁾ though the lowest energy has been established at a temperature of 0°C in pure water molecules, resulting in ice crystal formation (Fig. 2). It has been suggested by the author that the cage and hydrophobic effect might play dominant roles in the gel-formation processes of the polysaccharides.

On the basis of present considerations, it can be said that agarose molecules play a dominant role in the center of the tetrahedral cavities (cages) occupied by water molecules, the arrangement of which is very nearly a tetrahedral ice-like structure (Fig. 2) and should lead to a cooperative effect, stabilizing extended regions of ice-like water with hydrogen bonding on the surface of the polymer molecules, in which ring oxygen and hydroxyl groups (OH) may participate in hydrogen bonding with water molecules, and the more extended ice-like hydrogen bonding through water molecules may be achieved to form a cluster.³⁰⁾ Namely, polysaccharide gel formation may correspond to a total of the clusters.

Water molecules at low temperature around 0°C immediately changed into ice crystals upon the addition of some contaminant such as small dust particles. Such ice crystals may be established by the cage effect,²⁶⁾ which may lead to the lowest energy state of electrons, between water molecules through hydrogen bonding.³¹⁾ Furthermore, an amylose solution, for example, at a concentration of 1.0% (w/v), changed into a gel immediately under weak mechanical stimulation caused by the cage effect even at room temperature ($15\text{--}20^\circ\text{C}$).

Accordingly, the gel-forming polysaccharides, κ -carrageenan,^{1,2)} ι -carrageenan,³⁾ agarose (agar),⁴⁾ gellan gum,⁵⁻⁹⁾ curdlan,^{10,11)} amylose,¹²⁻¹⁴⁾ alginic acid,¹⁵⁾ or rice starch²⁵⁾ molecules, may adopt single-, double-, or multi-stranded conformations involving intra- and intermolecular associations, and

play a role in the center of the tetrahedral cavities leading and stabilizing the extended regions of ice-like water molecules with hydrogen bonding, and resulting in gelation or gelatinization. On the contrary, many other non-gelling polysaccharides, such as welan,^{6,7)} rhamsan,^{7,8)} S-657 gum,⁹⁾ schizophyllan,¹⁰⁾ rice amylopectin,^{13,14)} and galactomannans (locust-bean,^{15,17,19,20)} tara-bean,¹⁸⁾ and guar^{16,19,20)} gum), are free from intermolecular associations caused by the large kinetic energy and flexibility of the branched glycoside linkages even involving intramolecular associations in aqueous solution. Though the chemical structure of polysaccharides such as, welan, rhamsan, S-657 gum, schizophyllan or rice amylopectin is similar to that of gelling gellan gum, curdlan or amylose, gelation did not occur, but showed thermal stability for viscosity and dynamic viscoelasticity in aqueous solutions. This may be caused by involving intramolecular associations with hydrogen bonding and or van der Waals interaction in aqueous solution. Recently, we have proposed possible retrogradation mechanisms of rice starch.³²⁾ Though many investigations concerning the gelling properties of the polysaccharides have been done to discuss structure-function relationships, no one has established discussions at the molecular levels except the author. There is reasonable consistency in our investigations.¹⁻²⁵⁾ Thus, rheological analysis is one of the most significant methods to realize the structure-function relationships of polysaccharides in aqueous solutions.

Finally, the mode of intra- and intermolecular hydrogen bonding of agarose molecules, where the OH-4 of β -D-galactopyranosyl and OH-2 and ring oxygen groups of anhydro- α -L-galactopyranosyl residues take part in the secondary association with hydrogen bonding, has been supported by ¹H- and ¹³C-NMR spectroscopic analysis.³³⁾ ¹H- and ¹³C-NMR spectroscopy, in addition to rheological analysis, may give useful information to analyse the gelation mechanism of polysaccharides at the molecular level. It is expected that the era of the polysaccharides gels, which have received attention not only from the food industry, but also from many industries such as pharmaceutical, cosmetic and chemical engineering, will be coming in the

next century.

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多糖ゲルの構造原理

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著者はこれまで κ -カラギーナン, ι -カラギーナン, アガロース(寒天), ジェランガム, カードラン, アミロース, およびアルギン酸のゲル化機構を分子レベルで提出した。また, キサンタンガムとガラクトマンナン(ローカストビーングラム, タラビーングラムおよびグアーガム), キサンタンガムとコンニャクグルコマンナン, それに κ -カラギーナンとガラクトマンナン(ローカストビーングラム)の混合液のゲル化機構について分子レベルで提出した。さらに, 米澱粉の糊化および老化機構についても分子レベルで提出した。これらの多糖類は, 分子鎖内および間結合(水素結合, イオン結合, 静電気結合, またはファンデアールズ力等)を形成してゲル化すると考えている。上述の多糖類は0.1-1.0% (w/v)の低い濃度で水溶液を室温で劇的にゲルに変える。このような現象はいままで分子レベルで原理として理解されてない。水(H_2O)分子は 0°C で二つの水素と二つのローンペアーにより四つの水素結合を形成して正四面体を探り氷の結晶を形成するが, 多糖ゲルの水分子は室温でこのような正四面体を探っている。たとえば, 0.1% (w/v)のアガロース分子鎖は分子鎖内および間水素結合を形成し, 室温でも99.9%の水分子に正四面体の水素結合を形成させ, ゲルに変える。多糖類の分子鎖は水溶液で単鎖ラセン, 二重ラセン, または多重ラセンを採って水分子に水のような正四面体の水素結合を形成させるゲルの核としての役割を果たしている。多糖ゲルの形成にカゴ効果や疎水効果等が大きな役割を果たしている。