



Universality and specificity in molecular orientation in anisotropic gels prepared by diffusion method



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ABSTRACT

Molecular orientation in anisotropic gels of chitosan, Curdlan and DNA obtained by dialysis of those aqueous solutions in gelation-inducing solutions was investigated. In this diffusion method (or dialysis method), the gel formation was induced by letting small molecules diffuse in or out of the polymer solutions through the surface. For the gels of DNA and chitosan, the polymer chains aligned perpendicular to the diffusion direction. The same direction of molecular orientation was observed for the Curdlan gel prepared in the dialysis cell. On the other hand, a peculiar nature was observed for the Curdlan gel prepared in the dialysis tube: the molecular orientation was perpendicular to the diffusion direction in the outermost layer of the gel, while the orientation was parallel to the diffusion direction in the inner translucent layer. The orientation parallel to the diffusion direction is attributed to a small deformation of the inner translucent layer caused by a slight shrinkage of the central region after the gel formation. At least near the surface of the gel, the molecular orientation perpendicular to the diffusion direction is a universal characteristic for the gels prepared by the diffusion method.

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1. Introduction

Biopolymer-based hydrogels have widely been investigated because of their application in food industry and potential uses in biomedical and pharmaceutical fields. In most cases, aqueous solutions of biopolymers form hydrogels by addition of various cross-linking agents such as multivalent ions. For example, alginate, a polysaccharide mainly isolated from brown algae, makes a gel when its aqueous solution is mixed with multivalent cations such as calcium ions (Donati & Paoletti, 2009). In other cases, hydrogels without any cross-linking agents can be obtained when ionic biopolymer solutions are neutralized and self-assembled fibers or aggregates are formed. For instance, collagen, the major structural protein in the extracellular matrix, self-assembles into fibrils when its acidic solution is neutralized, resulting in the gel formation

without cross-linking agents (Forgacs, Newman, Hinner, Maier, & Sackmann, 2003).

Calcium-alginate gel as an example of hydrogels with cross-linking agents is often prepared by a diffusion method (or a dialysis method) (Draget, Smithrød, & Skjåk-Bræk, 2005). In the diffusion method, an aqueous solution of alginate in a dialysis tube is immersed in an aqueous solution containing calcium ions. Because of the semipermeability of the dialysis membrane, calcium ions flow through the membrane into the alginate solution and cross-link the alginate molecules, resulting in the formation of gel. The alginate gels by the diffusion method show a non-uniform alginate concentration distribution in the gels with the highest concentration at the dialysis tube and a gradual decrease in the concentration toward the center (Mørch, Donati, Strand, & Skjåk-Bræk, 2006; Skjåk-Bræk, Grasdalen, & Smithrød, 1989). Moreover, the alginate gels by the diffusion method are anisotropic showing birefringence due to the molecular orientation (Maki et al., 2009, 2011; Thiele, 1954). In the previous study we examined anisotropic structures of alginate gels in detail by birefringence and small-angle X-ray scattering (SAXS). The characteristics of the anisotropic alginate gels are summarized as follows (Maki et al., 2011). (1) Alginate molecules align circumferentially in a cylindrical gel, i.e., perpendicular to the

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direction of the calcium-ion diffusion. (2) The gel is isotropic when it is observed from its radial direction, indicating that the axis of symmetry of molecular orientation is parallel to the radial direction. (3) Rod-like structures with a radius of 6–7 nm were identified as corresponding to junction zones composed of lateral aggregates of alginate molecules by analysis of the SAXS profile of the gel, suggesting that the anisotropic structure is due to the orientation of the rod-like junction zones. (4) The gel is almost isotropic at the center and the degree of molecular orientation is higher near the surface.

Recently, Furusawa et al. (2012) reported that anisotropic collagen gels without any cross-linking agents were formed in the neutralization process. They prepared collagen gels by dialyzing acidic collagen solutions against neutral buffer solutions and studied the anisotropic gel structure by use of birefringence observation, small-angle light scattering and confocal laser scanning microscopy. It was shown that the chain orientation is perpendicular to the diffusion direction as in the case of the calcium-alginate gels, apparently indicating that the orientation direction may be universal for the diffusion-inducing gel formation.

Anisotropic gel formation by the diffusion method has also been reported for a semi-rigid synthetic polyelectrolyte (poly(2,2'-disulfonyl-4,40-benzidine terephthalamide, PBTD) cross-linked by calcium ions (Wu et al., 2011). In this case, unique molecular orientation behavior was observed. The gel was prepared in a rectangular cell consisting of two closely separated plates where one opposite sides are sealed with silicone spacers and another opposite sides are covered with dialysis membranes allowing multivalent ions to flow inside. The observation of the gel under polarizing microscope showed that the direction of molecular alignment was perpendicular to the flow of calcium ions near the surface but parallel to it near the center. This characteristic behavior implies that the orientation direction could depend on gel-forming systems.

In the previous studies, it was demonstrated that gels of various polysaccharides (Dobashi, Nobe, Yoshihara, & Yamamoto, 2004; Dobashi, Tomita, Maki, Chang, & Yamamoto, 2011; Lehtovaara, Verma, & Gu, 2012; Lin et al., 2010; Narita & Tokita, 2006; Nobe, Kuroda, et al., 2005; Nobe, Dobashi, & Yamamoto, 2005) and DNA (Furusawa, Minamisawa, Dobashi, & Yamamoto, 2007; Furusawa et al., 2010) obtained by the diffusion method were anisotropic showing birefringence. Curdlan, a microbial polysaccharide composed of β -(1,3)-glucosidic linkage, forms an anisotropic gel by immersing its alkaline solution in the dialysis tube in a copious aqueous solution of CaCl_2 (a dialysis tube method) (Dobashi et al., 2004; Nobe, Kuroda, et al., 2005). The similar anisotropic Curdlan gel can be also prepared by dialyzing Curdlan alkaline solution in the dialysis cell with cylindrical symmetry against aqueous CaCl_2 solution (a dialysis cell method) (Nobe, Dobashi, et al., 2005). The gel was sliced perpendicular to the cylindrical axis to prepare a disc-shaped specimen, and the specimen was observed under open and crossed nicols. In the observation under open nicols, the ring-shaped turbid layer was found in the gel (Dobashi et al., 2004; Nobe, Kuroda, et al., 2005). The observation under crossed nicols showed that the turbid layer and the region near the center of the gel are isotropic and the outermost region and the layer between the turbid layer and the isotropic central region are birefringent. The birefringence pattern of the anisotropic regions indicated radial or circumferential orientation of Curdlan molecules (Dobashi et al., 2004). Other kinds of gel specimens were prepared by slicing along several different planes parallel to the cylindrical axis and observed under crossed nicols, showing that the axis of symmetry of the orientation was along the radial direction (Dobashi et al., 2004).³

Anisotropic DNA gels induced by the diffusion method have been prepared in a different experimental setup (Furusawa et al., 2007, 2010). When aqueous solution of DNA sandwiched by a pair of circular cover glasses was immersed in a solution containing multivalent metal ions such as Co^{2+} , Cu^{2+} , and Al^{3+} , a gel film was formed due to ionic cross-linking between anionic DNA and multivalent cations. The DNA solution near the edge of the cover glass forms gel by the diffusion of metal ions immediately after the immersion and the gel membrane formed at the edge of the cover glass would act as a dialysis membrane (Furusawa et al., 2007). The gel film observed under crossed nicols exhibited a 'Maltese cross' birefringence pattern similar to the anisotropic alginate gels by the dialysis method, indicating radial or circumferential orientation of DNA molecules. A gel film of chitosan, a random copolymer composed of β -(1,4)-linked D-glucosamine and N-acetyl-D-glucosamine, has been prepared by the similar method (Dobashi et al., 2011). Chitosan dissolved in an aqueous acetic acid solution was sandwiched by a pair of cover glasses, followed by the immersion in a NaOH aqueous solution. In the neutralization process, chitosan molecules are insolubilized and form aggregates, followed by the formation of gel (Ladet, David, & Domard, 2008; Schatz, Pichot, Delair, Viton, & Domard, 2003). The obtained gel film exhibited a birefringence pattern similar to those of the other polysaccharides and DNA. The anisotropic gels of DNA and chitosan are hydrogels with and without cross-linking agents, respectively. The situation for the anisotropic Curdlan gel is rather complicated because Curdlan can form both types of gels: the gel formed by the cross-link with multivalent cations and that formed by the molecular aggregation in the neutralization process. In the dialysis process of Curdlan alkaline solution in CaCl_2 solution, the diffusion of Ca^{2+} into the Curdlan solution and the diffusion of OH^- out of the Curdlan solution occur simultaneously, inducing the gelation via cross-linking with Ca^{2+} and via the molecular aggregation. Because Ca^{2+} ions encounter Curdlan molecules at higher pH at the outer region of the dialysis tube and at lower pH at the inner region, respectively, it is expected that the Ca^{2+} cross-linked gel is formed near the surface of the cylindrical gel and the aggregation-induced gel is formed near the center.

In the previous studies, the direction of the molecular orientation has not exactly been determined for the anisotropic gels of Curdlan, chitosan and DNA. In the present study, the molecular alignment in these biopolymer gels by the diffusion method is investigated in order to clarify universal and specific aspects in the molecular orientation in the diffusion-induced gel formation.

2. Experimental

DNA purified from salmon milt with ca. 10 kbp was provided by Nippon Chemical Feed Co. Ltd., Japan. Chitosan (chitosan300, deacetylation ratio >80%) with average molecular weight $M_w = 1.1 \times 10^6$ was purchased from Wako Pure Chemical Industries Ltd., Japan. Two kinds of Curdlan samples were used: one with $M_v = 5.9 \times 10^5$ was from Wako Pure Chemical Industries Ltd. (CD1) and the other with $M_v = 2.1 \times 10^6$ was from Takeda-Kirin Foods Corp., Japan (CD2). These samples were used without further purification. Aluminum chloride, sodium tetra-borate, sodium hydroxide, calcium chloride and acetic acid of reagent grade were used. Milli-Q water was used for solvent.

In the preparation of the anisotropic gel of DNA (Furusawa et al., 2007, 2010; Furusawa, Minamisawa, Dobashi, & Yamamoto, 2009), DNA was dissolved in 20 mM sodium tetra-borate solution (pH 9.2)

³ In the previous study (Dobashi et al., 2004), the authors concluded that Curdlan molecules aligned radially in the cylindrical gel. However, their experiments

indicate that the axis of symmetry of the orientation is along the radial direction, but whether the direction of orientation is radial or circumferential cannot be identified from their data.

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