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Monitoring the Self-Assembly of Alginate Hydrogel Using Synchrotron Radiation Circular Dichroism

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Circular dichroism (CD) spectroscopy, traditionally used for studying the secondary structure of proteins and peptides [1], has not been yet established for studying polysaccharides (PSs). The current study has explored the potential of using CD in structural changes associated with PS hydrogelation. Certain PSs can change from a liquid to a gel when exposed to light, ions, or temperature. Alginate, an ecofriendly and nontoxic PS used in medicine and food, is especially known for forming hydrogels when mixed with polyvalent ions [2]. In the food industry, alginate serves as an additive to enhance texture and shape. This function is closely related to the conformational changes during sol-gel transitions, so structural analysis of alginate is helpful for maximizing its function as an additive.

Herein, CD experiments using synchrotron radiation (SR) light were carried out to investigate the structural changes associated with gel formation in the presence and absence of metal ions. Monovalent ions such as Na^+, K^+, and Li^+ did not induce hydrogel formation, whereas hydrogels were obtained by divalents (e.g., Ca^{2+}, Ba^{2+}, Cu^{2+}, and Zn^{2+}), as well as trivalent ion (e.g., Fe^{3+}). The cations exhibited different influences on the native conformation of alginate, suggesting variations in the assembly of the alginate chains in the presence of different ions. Additionally, discrepancies in CD spectra reflected that the formed hydrogels possessed diverse mechanical, physical properties, and morphology.

The formulation of hydrogels is an evidence of the self-assembly phenomeneon. Alginate chains are inter-connected, forming a cross-linked 3D-network structure that entraps water molecules, leading to hydrogel formation. Hence, to construct the self-assembly/gel phase diagram based on the CD observations, concentration-dependent experiments, varying alginate concentrations or polyvalent ion concnetrations (i.e., Ca^{2+}), were conducted using a CD spectrometer. The concentration of the polyvalent ion that causes no change in the CD spectrum of alginate or that self-assembly of alginate chains was terminated (**Figure 1**, left) was marked as the initiation of aggregation. As a result, a Ca^{2+} concentration of 1.56 mM, at 1.0 mg/ml alginate concentration, was referred to the transition concentration from the self-assembly to aggregation which is followed by hydrogel formation (**Figure 1**, right). This finding was reinforced by atomic force microscopy (AFM) imaging, which showed increased cross-linking and thichness of fibers at higher Ca^{2+} concentration (2.5 mM) (**Figure 2**).

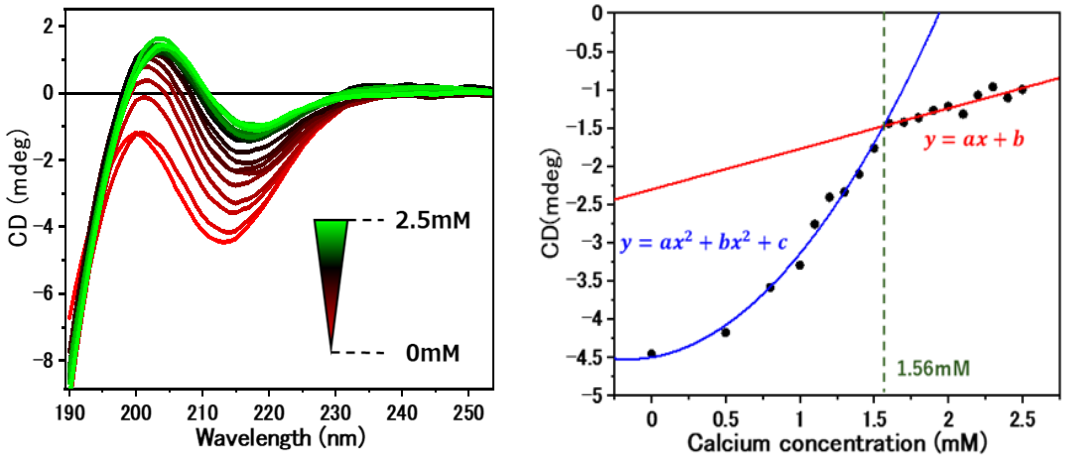

FIGURE 1. CD spectrum of alginate (1.0 mg/ml) as a function of Ca^{2+} concentrations (0.5 mM ~ 2.5 mM) (left), and self-assembly transition diagram based on CD observations (right).

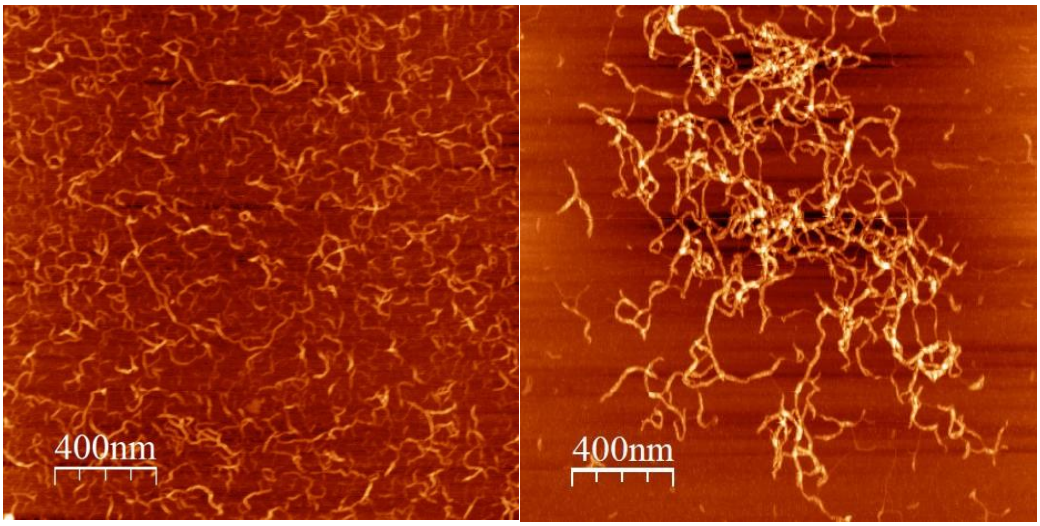

FIGURE 2. AFM images of the alginate-Ca^{2+} system with [alginate] = 1.0 mg/ml and Ca^{2+} concentrations of 1.0 mM (left), or 2.5 mM (right).

Ongoing investigations are underway to further explore the alginate-Ca^{2+} hydrogel system using SRCD, aiming to study the kinetics and thermodynamics of hydrogelation process.

REFERENCES

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