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Double-Metal Complexation of Heterogels Containing Cyanobacterial Polysaccharides

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ABSTRACT: Cryogenic-transmission electron microscopy of metal complexes with cyanobacterial polysaccharides, sacran, extracted from *Aphanothece sacrum* biomaterials reveals that Nd^{3+} complexes form networks composed of thick strings with a thickness ranging 10–20 nm while Fe^{2+} ones make dense entanglement of very thin strings. When Fe^{2+} and Nd^{3+} double complexation occurs, dense nanonetworks composed of thick strings with a thickness around 10 nm are formed. Next we prepared heterogels by crosslinking poly(vinyl alcohol) chains in the presence of sacran and investigated the effects of double complexation on metal sorption. The amount of Nd^{3+} sorbed into the heterogels in the 1 : 1 miscible solution of Fe^{2+} and Nd^{3+} is higher than that in the solution containing only Nd^{3+}, suggesting that Fe^{2+} assists the Nd^{3+} sorption. In addition, it is found that the amount of sorbed Nd^{3+} is much higher than that of sorbed Fe^{2+} even in the acidic condition of pH 2. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: cyanobacteria; gels; metal complexation; polysaccharides; rare earth metals

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INTRODUCTION

Cyanobacterial extracellular polysaccharides (ECP) adsorb heavy metal ions in the water to prevent toxicity and protect the cells themselves. In addition, it has been reported that polysaccharides secreted outside of the cells are crosslinked with metal ions to flocculate and precipitate them.[1,2] Thus, there is a strong relationship between the ECPs produced by cyanobacteria and metal ions adsorption. The structure and function of the ECP from the terrestrial jelly-like structure formed by cyanobacteria representing *Nostoc* genus have been previously reported.[3–5] However, their availability was limited because of the difficulty in artificial mass cultivation. On the other hand, *Aphanothece sacrum* which is a cyanobacterium with an abundant extracellular matrix (ECM)[6] is mass cultured in fresh water and can be grown in groundwater containing various metal ions. The ECP made by this organism adsorbs these ions to create a jelly ECM which protects the cells. In a previous study, we reported that sacran extracted from a jelly-like biomaterial of *A. sacrum* could adsorb multivalent metals ions.[7,8] Sacran has an extremely high molecular weight of 1.6×10^7 g/mol and has carboxylate groups at 22 mol % and sulfate groups at about 11 mol % to the sugar residues. It was demonstrated that sacran could form hydrogels by binding with trivalent metal ions at low ionic concentrations.[7–11] However, metal adsorption behavior of sacran in metal-ion miscible system which is a condition similar with the natural environment remains unknown. Therefore, in this study, a sorption behavior of the metal ions onto the sacran chains in a miscible system is investigated.

Here, we selected neodymium (Nd) from the critical elements, because Nd is gaining attention in high-performance magnets in motors of electric vehicle, elevator, and other motivity for transportation.[12] As the Nd magnet is an alloy including a high content of Fe, if these metals could be isolated and recovered from the magnets, then the Nd could be reused. Currently, the isolation method for recovering Nd as Nd oxide or a Nd salt compound uses strong acids,[13,14] and extraction methods for Nd using both liquefied Mg and high-heat processing (more than 650°C)[15] have been reported. However, there have been no reports of an Nd extraction from Fe at normal temperature. Therefore, evaluations of the sorption properties and quantitative determinations between Nd^{3+} and Fe^{2+} were carried out using sacran-containing hydrogels.

EXPERIMENTAL

Materials

Frozen samples of *A. sacrum* were gifted from Kisendou (Asakura, Japan) and used as received. Sodium hydroxide (NaOH), isopropanol, and glutaraldehyde (25% aqueous solution) were

Additional Supporting Information may be found in the online version of this article.

used as received (KANTO chemical co). Sodium alginate for comparison with sacran was used as received (KANTO chemical co). PVA [poly(vinyl alcohol)] for entrapment of polysaccharide chains was used as received (KANTO chemical).

Preparation of Heterogels

Heterogels composed of PVA networks with polysaccharide chains (sacran or alginate) were prepared as follows. A 1 wt % PVA solution of 100 mL and a 0.8 wt % polysaccharide solution of 100 mL were mixed with agitation to a homogeneous solution at pH 6. After agitation, glutaraldehyde (2 mol % to the PVA) was added to crosslink the PVA chains, and then the pH of the solution was adjusted to 2.4–2.5 by the careful addition of 0.1 M HCl to accelerate the acetyl reaction of the glutaraldehyde with the PVA via hydroxyls. The mixed solution was centrifuged to gather the air bubbles on the surface, and the solution was rested until the gels formed. Normal PVA gels crosslinked by glutaraldehyde were also prepared for comparison. The gels were then swelled in distilled water for 1 week while replacing the water for purification. The swelling degree of the gels was measured by the method shown below. Four block gels (size: 1 cm^3) in an equilibrated-swollen state were weighed. The degree of swelling was the mean of the weight ratio of four block gels before and after drying.

Phenol/Sulfuric Acid Assay

To confirm the polysaccharide chain entrapment into the PVA network, phenol/sulfuric acid assay was performed. A phenol aqueous solution (1 mL, 2 w/v %) was mixed into water dispersion of broken gels (1 mL) in a reaction tube, and then a concentrated sulfuric acid (probe assay grade, 4 mL) was added and agitated for 10 min to give a homogeneous solution. After the resulting solution was kept still for 15 min at room temperature, light absorbance of the solution at 485 nm was measured. Glucose was used as a external standard (concentration range; 0–200 ug/mL).

Metal Ion Sorption

Sorption in Individual Metal Ion Solution. Aqueous solutions of Nd^{3+} chloride at concentrations ranging between 10^{-5} and 10^{0} M were prepared just before the sorption tests. The heterogels were cut into $1 \times 1 \times 1$ cm^3 cubes ($n = 4$) and were immersed into individual concentration solutions of 10 mL in closed containers and shaken (100 strokes/min) for 4 days. After the Nd^{3+} was fully sorbed into each gel, the gels were taken and weighed, and the supernatant solutions of Nd^{3+} at each concentration were treated with HNO_3 to decontaminate any organic substances. The remaining Nd^{3+} ion concentration was finally measured by ICP (inductively coupled plasma) analysis using a Shimadzu ICPS-8100. Based on the Nd^{3+} concentration, the sorption ratio per gel weight and the sorption ratio per sacran negative charge (33 mol % to the sugar residues) were then calculated. When ferric (Fe^{2+}) chloride solutions of each concentration were prepared, deaerated water was used. The gels were placed in a Fe^{2+} solution of a 10 mL centrifuge tube (the cap was bored with a microscopic pin hole using a needle), and the centrifuge tube containing the gels and Fe^{2+} solution was packaged in a polyethylene bag with a zipper. The air in the bag was then removed by a vacuum pump, and nitrogen gas was filled to prevent oxidation from Fe^{2+} to Fe^{3+}. After the same sorption procedure of the Fe^{2+} to gels was performed, the residual concentration was measured by ICP in the same way. Furthermore, the pH effects on sorption were investigated, the hydrogels were presoaked for more than 5 days in each aqueous solution of the given pH, and then transferred to the Nd^{3+} or Fe^{2+} solution of the same pH, respectively. The evaluation of the amount of Nd^{3+} and Fe^{2+} ions sorbed was performed in the same manner as per the normal $NdCl_3$ and $FeCl_2$ solutions. The detail method for calculation of absorption ratio from the concentration value originated by ICP measurement is shown in Supplementary Information.

Sorption in Nd^{3+}/Fe^{2+} Mixed System. Solutions of 0.5 M $NdCl_3$and 0.5 M $FeCl_2$ were separately prepared and mixed to a volume of 10 mL, and distilled water was added until a 0.1 M mixed solution of Nd^{3+}/Fe^{2+} was obtained. Furthermore, the Nd^{3+}/Fe^{2+} mixed solution was prepared to various concentrations ranged between 10^{-5} and 10^{-1} M. In this preparation, deaerated water was used, and the air in the bottle was displaced with nitrogen gas as per the method above. The sorption test on the Nd^{3+}/Fe^{2+} miscible system was performed in the same manner as the Fe^{2+} sorption. In addition, the pH effect on the amount adsorbed by each gel was also investigated. The adjustment of the pH was performed by the following procedure. The Nd^{3+}/Fe^{2+} mixed solution whose concentrations were 0.316 mM in a 10 mL volume was prepared, and HCl was added, and the heterogels were stored in each solution for 4 days. How to measure the metal ion sorption ratio to the hydrogels was the same manner with those shown in the former subsection.

Cryogenic-Transmission Electron Microscopy

Cryogenic-transmission electron microscopy (Cryo-TEM) images were obtained with a JEOL JEM-2100 F (G5) operated at an acceleration voltage of 200 kV at a magnification of 100,000×. For specimen preparation, a thin layer of sample solution or dispersion was rapidly frozen to achieve sufficient cooling fast, so as not to rearrange the water molecules into a crystalline form. After a little solution was placed on an electron microscopy microgrid, the excess solution on the grid was drained off with filter paper, and the grid was immediately plunged into liquid propane maintained at around 100 K in an immersion cryofixation apparatus (Leica, Reichert KF 80 plunger). It was then placed in the compartment of a specially-designed cryotransfer system attached to the Cryo-TEM system. A helium stage was equipped with this system to keep the specimens at around 4.2 K, so that the structures formed in the solution could be observed in vitreous water as they are in solution.

RESULTS AND DISCUSSION

Cryo-TEM

Sacran was extracted from *A. sacrum* biomaterials by a previously reported method,[10,11] and the molar ratio of anionic groups to the sugar residues was 29.5 mol % (carboxylate: 17.0 mol %, sulfate: 12.5 mol %), which was almost the same value as the previous report. Sacran was a supergiant polyanion and then Ca^{2+} ions in the river where *A. sacrum* are living (concentration, 21 ppm) were condensed into the biomaterials of *A.*

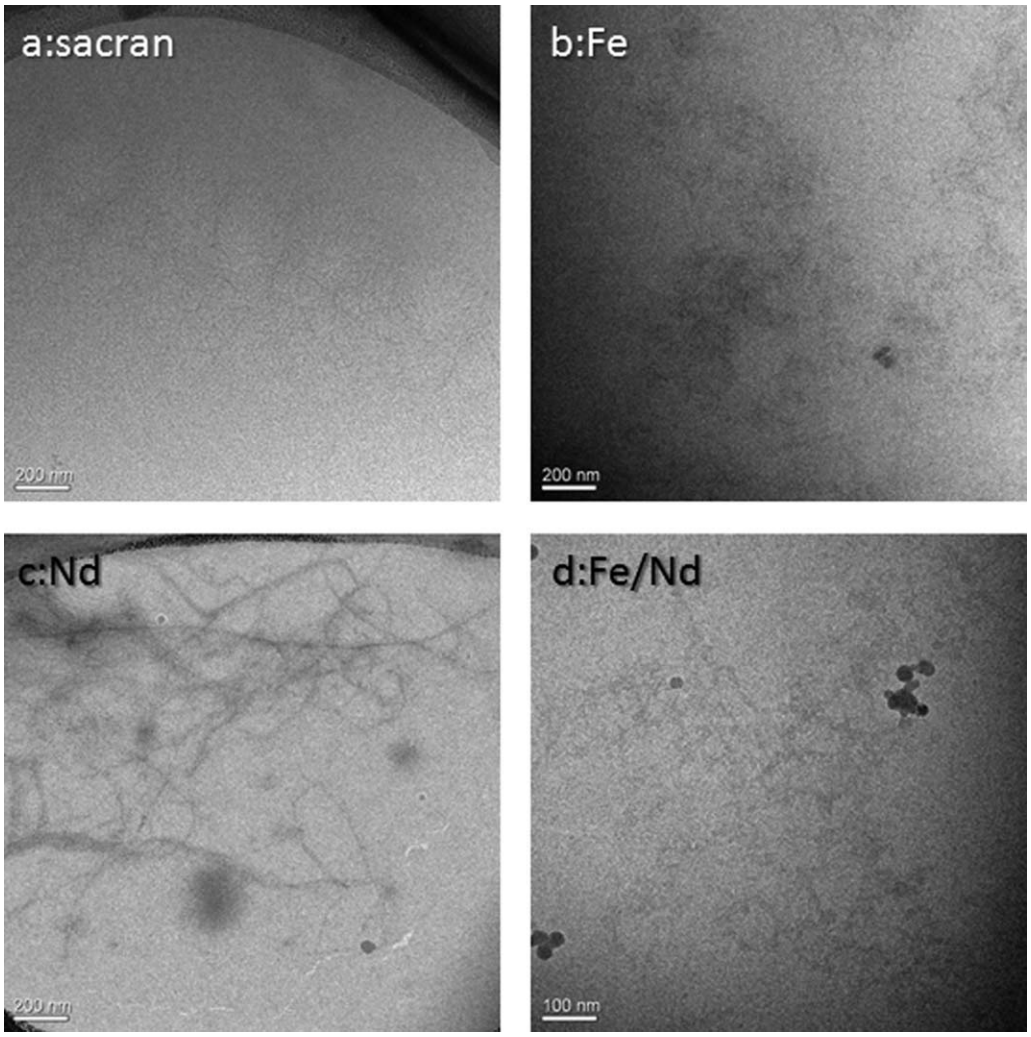

Figure 1. Cryo-TEM of supercooled samples. (a) normal solution of as-extracted sacran ($c = 0.1$ wt %), (b) dispersions for metal complexes of sacran with Fe^{2+}, (c) dispersions for metal complexes of sacran with Nd^{3+}, and (d) dispersions for metal complexes of sacran with Nd^{3+} and Fe^{2+}.

sacrum biomaterials (concentration, 270–400 ppm).[10,11] This suggests that the ECPs sacran interact with metal ions within a living organism. Then, we tried to mix a sacran solution of $c =$ 0.5 wt % with trivalent metal ion such as Nd^{3+} with low concentrations (0.1 mM), small fibers with a micrometer length were formed at once. However, the fibers were too thick to observe the nanostructure by electron microscopy. Then the concentration of sacran solution was reduced into $c = 0.1$ wt % and mixed with the same metal solution. No complexes were observed macroscopically but the viscosity of the solution was reduced slightly to make us to imagine the nanoconstruct formation. To confirm the nanoconstruct formation in various mixtures of sacran and metal ions, we made Cryo-TEM observation and the resulting images are shown in Figure 1. Although no clear nanoconstructs were seen in the normal sacran solution [Figure 1(a)], many nanofibrils with thickness less than 5 nm and length ranging between 10 and 50 nm were vaguely dispersed in the mixture of sacran chains and $FeCl_2$ [Figure 1(b)]. On the other hand, the complexes of sacran chains with Nd^{3+} showed a clear Cryo-TEM image of nanonetworks composed of thicker strings with thickness ranging 10–30 nm and micrometer scale length [Figure 1(c)]. In Fe^{2+} and Nd^{3+} miscible system, Cryo-TEM image revealed the formation of dense nanonetworks composed of strings thicker than those in only Fe^{2+} but thinner than those in Nd^{3+} [Figure 1(d)]. Such a dense nanonetwork might be effective on enhancing the metal sorption into sacran chains. The shape change of nanoconstructs will be discussed later after the metal sorption studies.

Heterogel Preparation

We selected PVA as nonionic chains, which were easily crosslinked by glutaraldehyde to form tough hydrogels. Tough heterogels composed of PVA networks and sacran chains (sacran/PVA gels) were successfully prepared by adding glutaraldehyde to a mixture of PVA and sacran at pH 2–3 (Figure 2). Heterogels composed of PVA networks and alginate chains (alginate/PVA gels) and normal PVA gels without polysaccharides were also prepared for comparison. We did not confirm the formation of hydrogels following the reaction of the sacran or alginate chains with glutaraldehyde in this condition, but a slight

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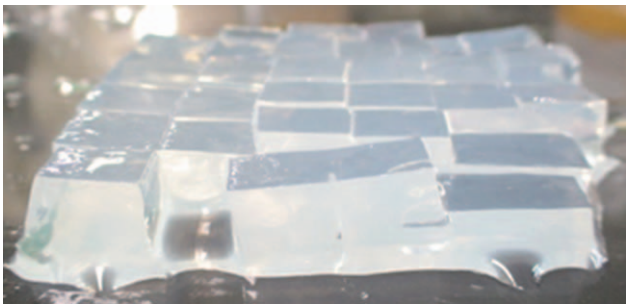

Figure 2. Schematic illustration of heterogels composed of PVA networks and polysaccharide chains (sacran or alginate) and partial structure of sacran is shown at the bottom. Inset: photographs of the heterogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

increase in viscosity was observed. This finding indicates that the reaction of glutaraldehyde with the PVA chains occurs preferentially to the reaction with the polysaccharides. We confirmed the entrapment of sacran in PVA networks by phenol/sulfuric acid assay[16] of the heterogels as illustrated in inset at the bottom of Figure 2. As a result, it was found that 96.6% to in-fed amount of sacran chains were entrapped into the PVA networks. As a result of the swelling degree measurement of these gels, the sacran/PVA gel swelled to 22-fold of the dry weight, the alginate/PVA's swelled by 18-fold, and the PVA gel swelled by 5-fold, respectively. These data showed that the sacran chains had stronger effects on the increase in the water absorption of the gels than alginate chains, presumably due to the sacran chains have a higher water retention capacity than alginates as shown in the previous paper.[7]

Metal Complexation

Separated Solutions. Heterogels composed of PVA networks with polysaccharide chains (sacran or alginate) were used to evaluate the amount of Nd^{3+} sorbed as compared with Fe^{2+} ions into the gels (Figure 3). In the case of the sorption test of Fe^{2+} ions, degassed water was used to prevent oxidation, and substitution from air to nitrogen was used. Thus, precipitation by oxide generation was not observed in the solution containing Fe^{2+} ions for 4 days. On the other hand, oxide generation was observed in the absence of nitrogen substitution within 12 h. As a result of heterogel immersion into $NdCl_3$ solution, the gel was reduced in size and lost the transparency, which suggests the complex formation of sacran chains with metal ions inside of the gels. To quantify the sorption ratio of Nd^{3+} and Fe^{2+} ions into the gels, the concentration decrease of each metal ion in

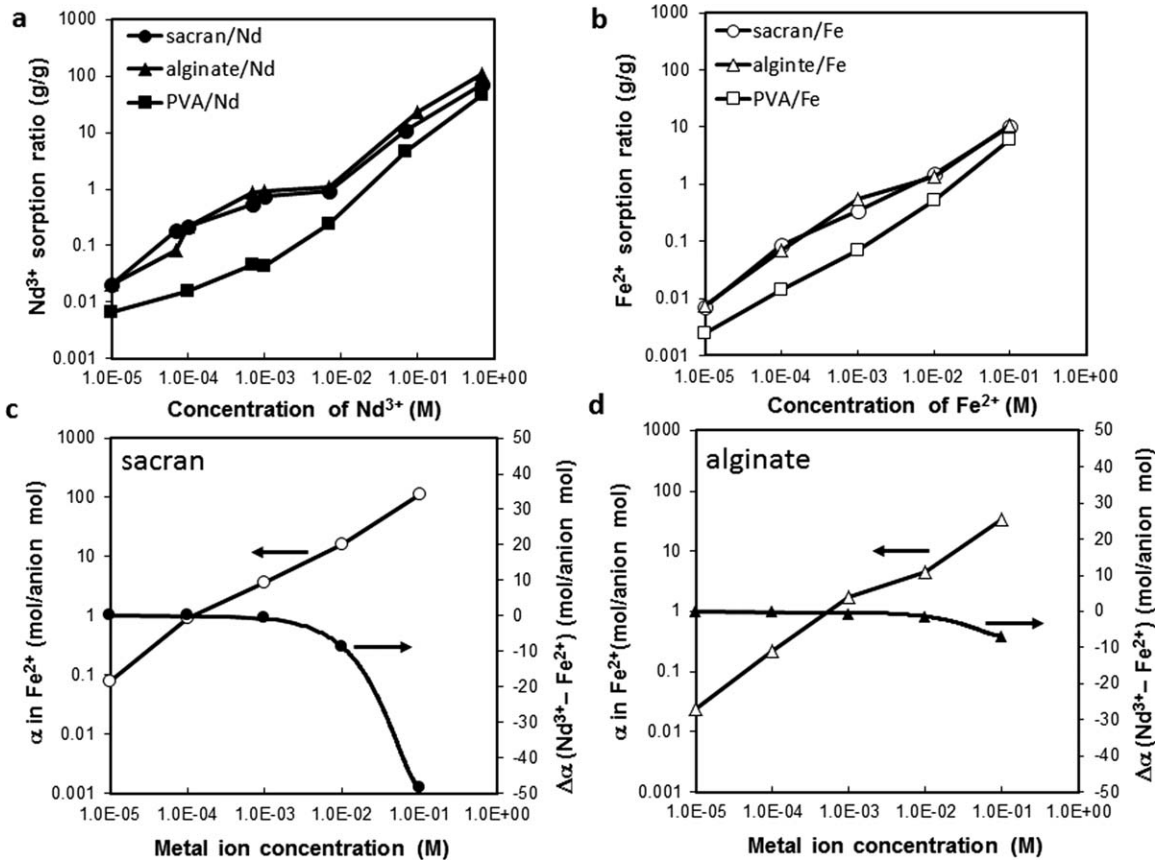

Figure 3. Changes in sorption ratios in heterogels composed of PVA networks and polysaccharide chains as a function of the concentration of metal ions. PVA gels containing no polysaccharides are also shown for comparison. The sorption weight ratios are shown in terms of (a) Nd^{3+} and (b) Fe^{2+}. (c) The sorption molar ratio, α, of metal ions to polysaccharide anions into heterogels composed of PVA networks and sacran, and difference between α values in Nd^{3+} and Fe^{2+}, $\Delta\alpha$, was shown together. (d) α to polysaccharide anions into heterogels composed of PVA networks and alginates, and $\Delta\alpha$ between Nd^{3+} and Fe^{2+} was shown together.

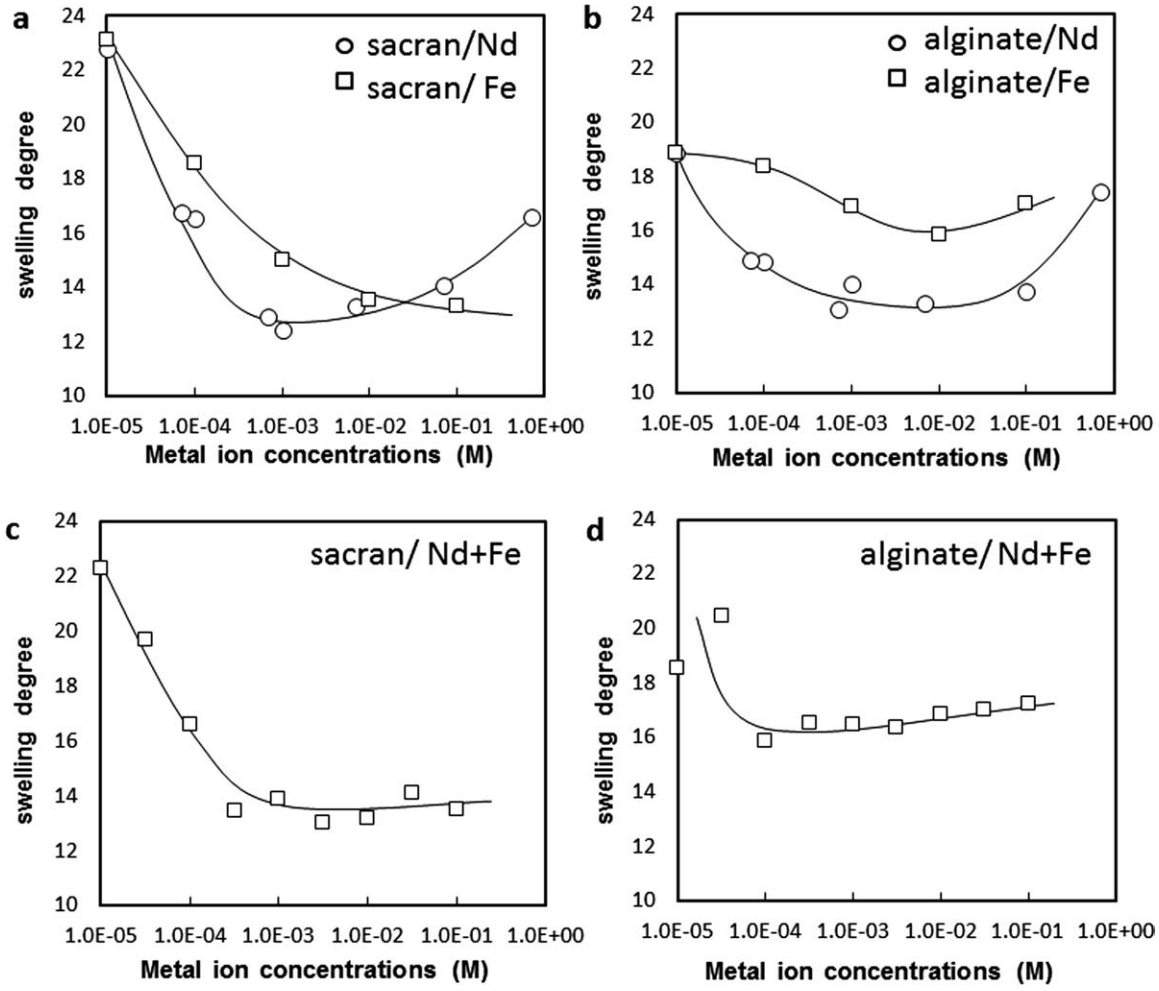

Figure 4. Metal ion concentration dependence of swelling degree of the heterogels. (a) Sacran/PVA gels in the separated solution. (b) Alginate/PVA gels in the separated solution. (c) Sacran/PVA gels in the Nd^{3+}/Fe^{2+} mixed solution. (d) Alginate/PVA gels in the Nd^{3+}/Fe^{2+} mixed solution.

the supernatant was measured by ICP emission. Weight ratios of the Nd^{3+} and Fe^{2+} sorption per 1 g of polymer components, such as polysaccharide chains or PVA networks, were then calculated [Figure 3(a, b)].

The metal sorption ratio in every metal/hydrogel systems increased with increasing the metal ion concentrations, which was a widely-known phenomenon in metal ion sorption. The sorption ratio of both Nd^{3+} and Fe^{2+} to the PVA gels containing no polysaccharides was lower than those of the gels containing either polysaccharide, which indicated that the polysaccharide chains were effective on enhancing the metal ion uptake. Especially, at a concentration of 7×10^{-1} M, the effects of polysaccharides chains looked very small in this logarithmic plot [Figure 3(a)], but in fact the sorption ratios of the heterogels containing sacran and alginate chains were 0.71 g/g [38 mg (Nd^{3+})/53 mg (dry gel)] and 1.09 g/g [68 mg (Nd^{3+})/62 mg (dry gel)]which were much higher than that of the PVA gels without polysaccharides 0.46 g/g [24 mg (Nd^{3+})/110 mg (dry gel)]. Then, the increased value of Nd^{3+} sorption ratio by polysaccharides addition into PVA networks was regarded as more than 0.25 g/g. If the dry powders of sacran were added into Nd^{3+} solution with a concentration range from 0.02 M to 0.2 M, the sorption ratio ranged between 0.05–0.1 g/g. As a consequence, the sacran addition method using the heterogels composed of PVA networks was very effective in the sorption of a large amount of Nd^{3+}. Sacran chains adsorbed Nd^{3+} ions, owing to a large amount of carboxylate and sulfate ions to form the gels when the dry powders of sacran were added into Nd^{3+} solution. However, the dense layer of sacran/Nd^{3+} complexes was created on the gel surface due to efficient Nd^{3+}-mediated association of sacran macromolecular chains.[8] The dense layer could block the ion diffusion into inner portion of the gels, to reduce the Nd^{3+} sorption ratio. In the heterogels, it can be considered that the chain association was restricted by PVA networks to inhibit the surface dense layer and to increase the sorption ratio. A difference in the sorption ratio between heterogels containing sacran and alginate chains was hardly ever observed. In Fe^{2+} uptake into the heterogels containing either polysaccharide, the sorption ratio increased almost monotonically with an increase in metal ion concentration and their increasing rate was similar with the PVA gels without polysaccharide in entire concentration range. In both metal ions, the sorption ratios into the heterogels containing polysaccharides

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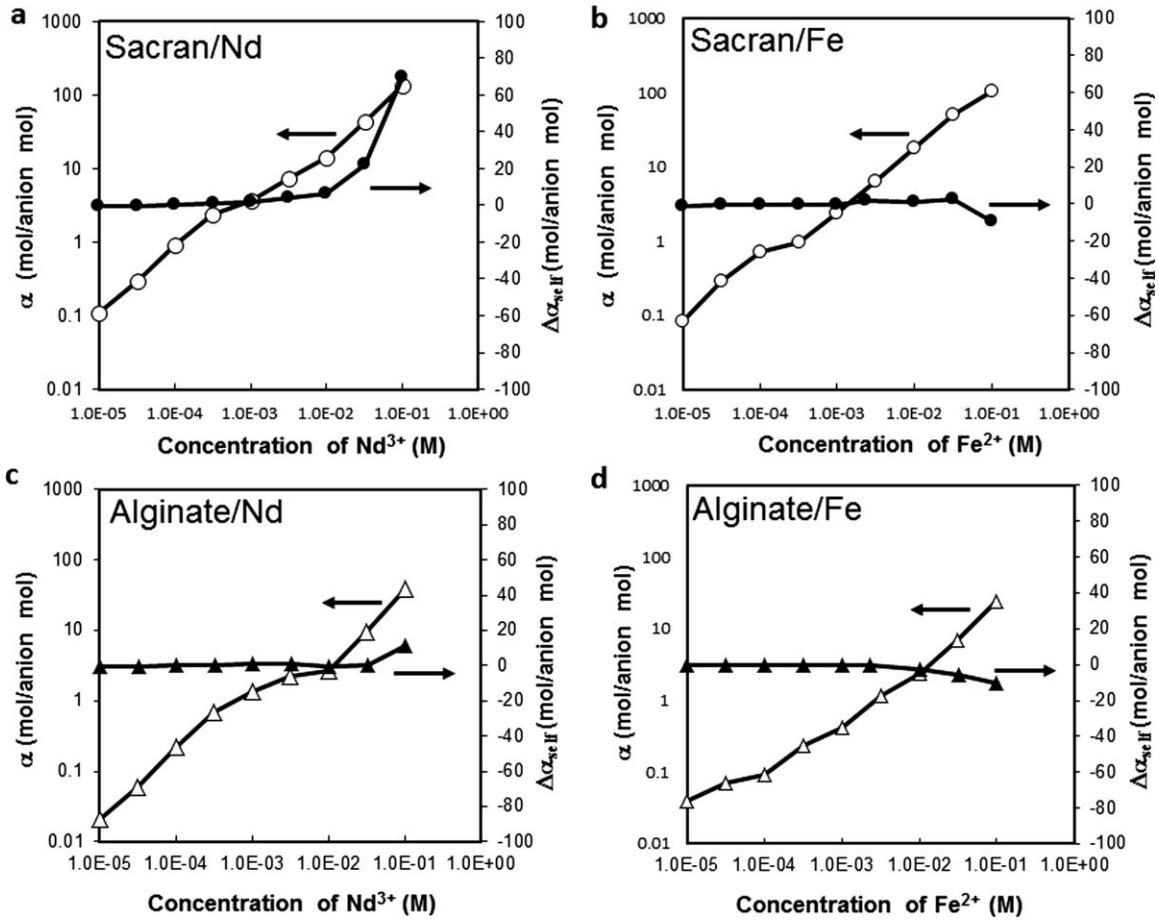

Figure 5. The sorption molar ratios, α, of metal ions in the miscible systems to polysaccharide anions in the heterogels composed of (a) PVA networks and sacran in Nd^{3+} sorption, (b) PVA networks and sacran in Fe^{2+} sorption, (c) PVA networks and alginate in Nd^{3+} sorption, and (d) PVA networks and alginate in Fe^{2+} sorption. In (a) and (c), α differences, $\Delta\alpha_{self,}$ of Nd^{3+} between systems of a Nd^{3+} solution without Fe^{2+} and a Nd^{3+} miscible system with Fe^{2+} were shown together. In (b) and (d), $\Delta\alpha_{self,}$ of Fe^{2+} were shown together.

chains were higher than PVA hydrogels without them, suggesting that the polysaccharide anions drew metal ions into the gels.

If the anions of polysaccharides were the origin of attraction force for metal ions, the multivalent metal ions should crosslink the polysaccharide chains. The anions can draw the metal cations through Coulombic interaction and bind to them through additional interactions such as metal coordination with hydroxyls and ethers of sugar residues. Figure 4(a, b) show the metal ion concentration dependence of swelling degree of the heterogels. The swelling degrees of the heterogels at a metal ion concentration of 10^{-5} M were a bit higher than those of as-prepared heterogels. In such a low concentration, very small amount of metal ions bound to polysaccharide chains and not crosslinked them when incomplete binding of a monovalent $[NdCl_2]^+$ ion occurred, instead, counter anions of Cl^- raised osmotic pressure of the heterogels to increase the swelling degree. Between 10^{-5} M and 10^{-2} M, the swelling degree decreased with an increase in metal ion concentration due to the ionic crosslinking of polysaccharide chains. On the contrary, the swelling degree increased slightly again with an increase in Nd^{3+} ion concentration ranging over 10^{-2} M, presumably due to the osmotic pressure gain by Nd^{3+} binding to nonionic PVA networks accompanied by Cl^- osmosis.

To compare the polysaccharide capability to bind with Nd^{3+} and Fe^{2+} ions, the data from Figure 3(a, b) were converted into molar ratios, α, of metal sorption binding to the molar ratio of polysaccharide anions: 29.5 mol % to the sugar residues for sacran anions, and 100 mol % for alginate [Figure 3(c, d)]. As shown in Figure 3(c), Δα values (Nd^{3+}-Fe^{2+}) in the sacran/PVA gels became negative at metal ion concentration over 0.001 M, indicating that a higher amount of Fe^{2+} sorbed to sacran chains in the heterogels. On the other hand, the alginate/PVA gels did not show very low Δα value. As a result, the most important finding in this experiments was a higher value of α in Fe^{2+} at 10^{-4} M than the stoichiometric ratio of $\alpha = 0.5$ (2 anions/1 Fe) [Figure 3(c)]. In the same concentration for the PVA/alginate gels, metal ions did not attain the stoichiometric ratio [Figure 3(d) shows around $\alpha = 0.2$], and then it was considered that osmotic pressure originated from the concentration difference between outside and inside of hydrogels was too small for metal ions to diffuse effectively into the heterogels. The reason for this higher sorption ratio of sacran when compared with alginate, which also possesses anions to a high degree (all monosaccharides have carboxylic acid) may be hypothesized as follows; as the molecular weight of sacran is about 100-fold greater than

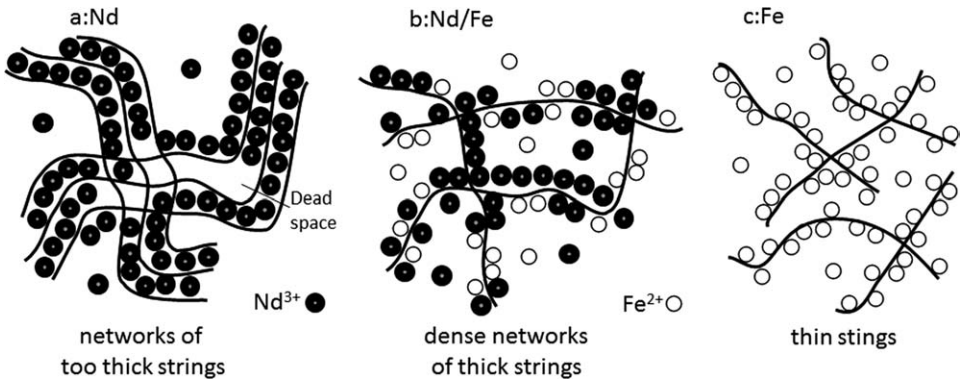

Figure 6. Schematic illustration of nanostructure formation mechanism in sacran-metalcomplexation system. (a) Nd^{3+} complex, (b) Nd^{3+} and Fe^{2+} double complex, (c) Fe^{2+} complex.

that of alginate, the number of negative charges per monomer chain is about 30-fold greater than alginate, and thus sacran could sorb ions more efficiently. Over 10^{-3} M, both heterogels showed higher sorption ratios for metal ions than the stoichiometric ratio, suggesting that not only adsorption but also absorption of metal ions into the heterogels should occur.

Mixed Solutions. The molar ratio of metal sorption in a Nd^{3+}/Fe^{2+} miscible system (each meal ion was at a 1:1 molar ratio) was investigated. Figure 5(a) shows that the molar ratios of Nd^{3+} sorption into the PVA/sacran gels in the Nd^{3+}/Fe^{2+} miscible system were higher than those in the solution without Fe^{2+}. On the other hand, the molar ratios of Fe^{2+} sorption into both heterogels in the miscible system were similar values with those in the separated solution [Figure 5(b)]. The similar tendency was observed in the alginate/PVA gels [Figure 5(c, d)]. To discuss the enhanced sorption of Nd^{3+} into the PVA/sacran gels in the miscible system, we discuss using the Cryo-TEM images (Figure 1) and the illustration of Figure 6, revealing the formation of dense nanonetwork composed of thick strings in the presence of Nd^{3+} and Fe^{2+}. Ionic binding of Nd^{3+} to sacran may induce the chain condensation to form the bundles. The numbers of sacran chains composing the too thick bundles with 10–20 nm thickness could be simply estimated as 400–1600 by the thickness of single sacran polysaccharide chains, 0.5 nm. The drastic binding with many anionic sites should induce a difficulty of the complete chain organization inside of the polysaccharide bundles and finally to desolvate them with remaining the unbound sites (dead space) as shown in the illustration of Figure 6(a). The formation of dead space should be inferred from the lower sorption ratio of Nd^{3+} than Fe^{2+} [Figure 3(c)]. Although further intrusion of Nd^{3+} into the dead space of the particles was difficult, Fe^{2+} with a smaller hydration size (ca. 600 pm)[17] than Nd^{3+} (ca. 900 pm)[17] could complex with the sites of the dead space. This Fe^{2+} complexation can ravel the too thick bundles at some extent and the formation of denser nanonetworks composed of thick strings with a thickness around 10 nm as seen in Cryo-TEM [Figure 1(b)], which is illustrated in Figure 6(b) The disorganization may be due to the formation difficulty of chain organization for Fe^{2+}/sacran system as seen in Cryo-TEM image [Figure 1(c)], which is illustrated in Figure 6(c).

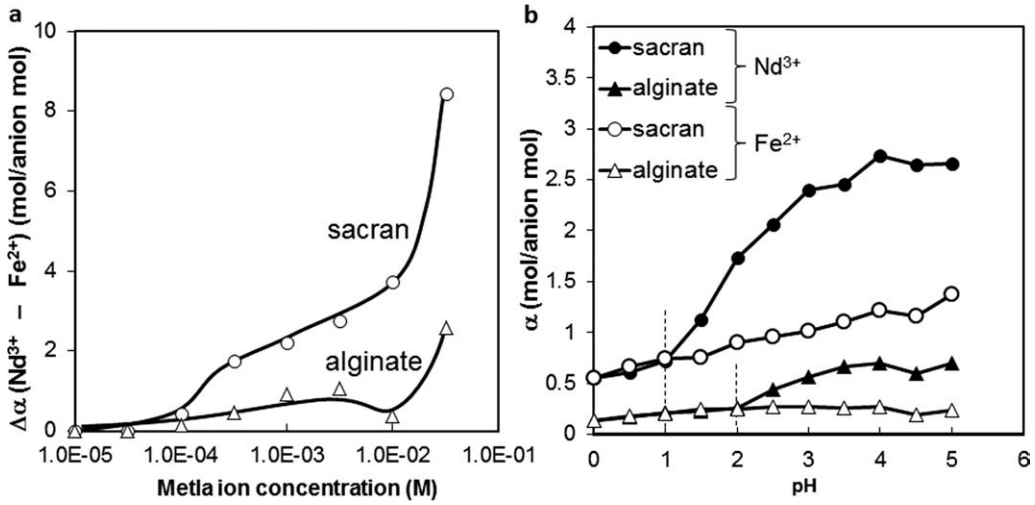

Figure 7. (a) Concentration dependence of $\Delta\alpha$ values between Nd^{3+} and Fe^{2+} ions sorbed into the heterogels in a one-to-one miscible system under an acidic condition (pH~3). (b) pH dependence on α of Nd^{3+} and Fe^{2+} into heterogels composed of PVA networks and polysaccharide chains. The concentration of Nd^{3+} was 0.316 mM.

The swelling degrees of the sacran/PVA gels in the miscible system showed the similar value as those in the solution of Nd^{3+} alone [Figure 4(c)] but kept the minimal value regardless of the further increase in the ionic strength (metal ion concentration). This phenomenon indicates that the structure by double complexation of both Nd^{3+} and Fe^{2+} in the miscible system was more stable than the structure containing the dead space in the separated Nd^{3+} solution. In alginate, the swelling degree of the heterogels in the miscible system showed the intermediate values between those of the heterogels in each metal ion solution [Figure 4(d)].

Sacran and alginate both have carboxylic acid groups as anionic sites, and then the pH was very effective on the control of the cation binding ability. The pH effects of metal binding to heterogels were investigated at a metal ion concentration of 3×10^{-4} M (pH $\sim$ 3) which was the lowest concentration to show Nd^{3+}-sorption selectivity of the sacran/PVA gels [Figure 7(a)]. According to Figure 7(a), α of Nd^{3+} by the sacran/PVA gels at acidic pH values was much higher than that of the alginate/PVA gels, showing α for both Nd^{3+} and Fe^{2+} greater than the theoretical adsorption value (0.33) under pH 1. It was speculated that the adsorption capability of the alginate molecule was reduced by the protonation of the carboxylic residue which seemed to adsorb metal ions. On the other hand, sacran could maintain its adsorption sites due to the presence of the sulfate groups. The selective sorption by several systems could not be confirmed under a pH value of 1. In addition, α difference between Nd^{3+} and Fe^{2+} by the sacran/PVA gels increased with a pH increase from pH 1 [Figure 7(b)]. On the other hand, in the case of alginate/PVA gels, α difference between Nd^{3+} and Fe^{2+} increased over pH 2. Then, sacran chains have a special advantage in a pH range between pH 1 and 2 where various metal alloys were dissolved. For sacran, it was hypothesized that the reason for this increase of selectivity under very acidic conditions was the effect of the sulfate group.

From this result, we speculated that the specific sorption of Nd^{3+} might be attributed to a binding site formed between the sacran molecular chains, but the selectivity of Nd^{3+} sorption in the range over $\alpha = 1$ means that the inner environment of the heterogels also has some mechanism for Nd^{3+} selection. Rare earth metals are commonly used as composites with another metal, and one can surmise that an isolation technology to recover each metal would be significant and useful. We will continuously explore the optimum conditions to improve the selectivity and efficiency of sacran-related composites.

CONCLUSION

We investigate double complexation behavior of Nd^{3+} and Fe^{2+} with cyanobacterial polysaccharides, sacran, which is a supergiant polyanions with an M_w ranging over 10^7 g/mol, extracted from the biomaterials of *A. sacrum* living in the river containing various metal ions. Cryo-TEM images of metal complexes with sacran reveals nanonetwork formation of the double complexes. In hetero-hydrogels composed of PVA networks entrapping the sacran chains, Nd^{3+} and Fe^{2+} were both sorbed highly and Fe^{2+} assisted the Nd^{3+} sorption. Furthermore, experiments on the pH dependence of the metal sorption showed the selective sorption of Nd^{3+} under pH over 1 in the sacran/PVA heterogels. The present finding implies the possibility of cyanobacterial polysaccharides with a thick jelly ECM for the important resource of metal-selection materials. Ln-based materials are becoming increasingly important both in terms of research activity[18] and their use in commercial products such as optical materials,[19] ion conductors,[20] microelectronics,[21] and ferromagnetic materials composed of Nd.[22] These Ln are trivalent cations in acidic solution from which these metals are required to recover. Then, the metal sorption using heterogels sacran leads to the important field of Ln-recovery from industrial waste and treated solutions.

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