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Metal Coordinative-crosslinked Polysaccharide Nanogels with Redox Sensitivity

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Metal coordinative-crosslinked nanogels were prepared by introducing a metal–ligand to a hydrophilic polysaccharide. The redox couple of Co(II) and Co(III) was utilized for construction of a redox-sensitive nanogel system. This is a new method for the preparation of hybrid nanogels with dual network structures of both physically and coordinative-crosslinking points.

Metal-complexing and metal-containing polymers are fascinating research targets, particularly because such materials have unique properties from their individual organic and inorganic components.[1] These polymers offer a wide variety of applications ranging from filtrations to catalysis. The coordinating ability of the metal within the polymer chain allows application of these materials to environmental metal sensing and as building blocks for supramolecular structures.[2]

Here, a new method for the preparation of coordinative-crosslinked nanogels by conjugating a metal ligand to a polysaccharide is reported. Nanogels are nanometer-sized hydrogel nanoparticles (<100 nm) with three-dimensional networks of crosslinked polymer chains. They have attracted growing interest over the last several years because of their potential for applications in biomedical fields, such as drug delivery systems and use in bioimaging.[3] Previously, we have reported that hydrophobized polysaccharides such as cholesterol-bearing pullulan (CHP, Figure 1) form stable amphiphilic nanogels in water by self-association of hydrophobic groups, which form physically crosslinked points.[4] CHP nanogels trap proteins and show molecular chaperone-like activity. Furthermore, CHP nanogels are useful for protein delivery applicable to cancer vaccines and cytokine therapy. Various intermolecular forces other than hydrophobic interactions have been utilized as a driving force in the preparation of physically crosslinked nanogels. For instance, nanogels have been prepared through electrostatic interactions[5] and through host–guest interactions with cyclodextrin.[6] Photoresponsive nanogels based on a spiropyrane-modified pullulan[7] and pH-responsive nanogels comprising ethylenediaminetetraacetic acid-bearing chitosan have also been reported.[8] Nanogels utilizing a wide range of intermolecular forces show great potential for the preparation of novel stimulus-responsive nanogels.

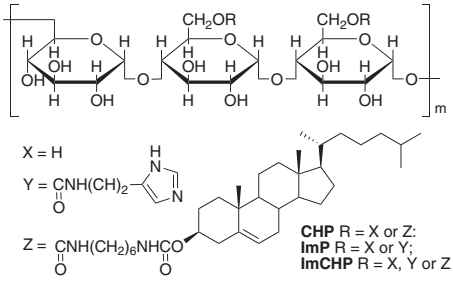

Figure 1. Structures of cholesterol- and/or imidazole-bearing pullulan.

The objective of this study is to develop a novel nanogel utilizing metal coordinative interactions for crosslink formation. As a metal ligand, imidazolyl groups were conjugated with pullulan or CHP (Figure 1). The imidazolyl group has a high affinity for divalent transition-metal ions to form a complex. The complexation of more than one ligand group per metal ion enables joining together two or more polymer chains at a metal-centered crosslink. This paper reports the first attempt to prepare metal coordinative-crosslinked nanogels with redox sensitivity by using redox coupling of Co(II) and Co(III), though macrogels of the metal-containing polymers[1] and coordination polymeric gels[9] have been extensively studied.

CHP, which was substituted with 1.1 cholesteryl groups per 100 glucose units of the parent pullulan ($M_w = 1.0 \times 10^5$), was synthesized as reported previously.[10] Imidazole-conjugated pullulan or CHP (ImP or ImCHP, respectively) were prepared by a two-step reaction: activation of hydroxy groups of pullulan with 4-nitrophenylchloroformate[11] and then reaction of the resultant nitrophenyl-substituted polysaccharides with histamine.[12]

The conjugation of imidazole was confirmed by the FT-IR spectrum for the products, which indicated formation of carbamate linkages (1700 and 1550 cm^{-1}) and the disappearance of the 4-nitrophenylchloroformate peak (1765 cm^{-1}). The ^{1}H NMR spectra (DMSO-d_6) showed distinctive peaks: the anomeric proton of the pullulan glucopyranosyl ring at 4.70 ppm, 2-(1*H*-imidazol-4-yl)ethyl group at 2.65–3.10 ppm for the ethyl group, and 7.54 ppm and 6.54 ppm for the aromatic imidazolyl group. The degree of substitution of imidazole coupled to the polysaccharides per 100 glucose units was quantitatively determined using the integrated areas of glucopyranosyl rings and the imidazolyl group to be 23 and 21 for ImP and ImCHP, respectively. Nanogels were prepared in pure water or buffer (100 mM HEPES, 100 mM NaCl, pH 7.5) according to an established protocol.[10,12]

The interactions of metal ions with the imidazolyl groups in ImP and ImCHP were evaluated using Cu(II) ($CuCl_2 \cdot 2H_2O$), which has the highest binding affinity with a wide variety of ligands in the Irving–William series. In the absence of the ligands, Cu(II) existed primarily as $[Cu(H_2O)_6]^{2+}$, which gave rise to a broad and weak absorption band centered at approximately 800 nm in the UV–vis spectra resulting from the d–d transition for Cu(II). In the presence of ligands (4-methylimida-

zole as a control compound, ImP or ImCHP), λ_{max} for the Cu(II) d–d transition became more prominent and shifted to 640 nm, reflecting the complexation of ligands and metal ions. In general, metal–ligand interactions are much enhanced when the ligand is placed on the polymer chain rather than in aqueous solution. In our case, the metal binding with ImP and ImCHP was also slightly enhanced compared with that of 4-methylimidazole dissolved in water, while the complexation of ImP and ImCHP was comparable under the current conditions (Figure S7).[12]

The above results indicate that imidazolyl groups grafted on polysaccharide act as ligands to Cu(II). However, such a complex did not induce any association of polymers. This is due to the intramolecular metal–ligand complexation or ligand-exchange reaction of the complex following the deformation of the crosslinking point. Subsequently, the labile metal complex was replaced with an inert complex by utilizing cobalt ions. Cobalt complexes are extensively studied, and the redox of the central metal ion can be controlled easily. In general, the kinetic stability of the cobalt complex was changed by its oxidation state.[13] Regarding Co(II), the complexes with nitrogen-donor ligands are kinetically labile. To the contrary, Co(III) complexes are kinetically inert. Thus, the redox couple of Co(II) and Co(III) could offer the preparation of a redox-sensitive nanogel.

Based on size-exclusion chromatography, multiangle laser-light-scattering (SEC-MALS) measurements,[12] the molecular weight (M_w) of the ImCHP was estimated to be 5.3×10^5 (Table 1, Entry 1). This value agrees with the M_w of the CHP nanogel (5.5×10^5), consisting of 5–6 CHP macromolecules. From these results, the nanogel formation of ImCHP was confirmed. Since the Co(II)-complex is kinetically labile, the M_w was not increased upon addition of Co(II) ($CoCl_2 \cdot 6H_2O$) to the ImCHP nanogels, (Table 1, Entries 2 and 3). In contrast, when the Co(II) was oxidized to Co(III) by the addition of 0.3 w/w % H_2O_2 for 30 min, the M_w significantly increased because of the association of ImCHP via the Co(III) complex formation. The *z*-average root-mean-square radius of gyration (R_g) of the ImCHP in the presence of Co(III) was determined to be ca. 40 nm. The R_g was comparable to that of ImCHP in the absence of cobalt ions. These results indicate that the physically crosslinked ImCHP nanogels were crosslinked by coordinative interactions with Co(III) to form inter-nanogel crosslinking or intra-nanogel crosslinking (Figure S8).[12] In the case of ImP, the M_w also increased approximately twofold after addition of Co(III), suggesting that the crosslinking with the metal ion was also formed to give nanogels (Table 1, Entries 6–8), whereas the M_w of ImP is much lower than unmodified pullulan probably due to hydrolysis during the synthesis.

To obtain further information on the crosslinking structure, interactions with β-cyclodextrin (β-CD) were investigated. When CHP nanogels were added to β-CD, the cholesteryl groups and β-CD formed an inclusion complex, and the CHP nanogels dissociated into single macromolecules. The ImCHP also gave a peak, which corresponds to a nonaggregated complex of ImCHP–CD (Table 1, Entry 4) after 30 min of the addition of 3.0 mM β-CD. This was almost the same molecular weight as the CHP monomer ($M_w = 1.0 \times 10^5$), indicating that the ImCHP nanogels also dissociated in the presence of β-CD. However, ImCHP nanogels that were crosslinked with Co(III) slightly swelled without dissociation, even in the presence of β-CD (Table 1, Entry 5). This suggests that ImCHP formed dual network nanogels with physically crosslinked and coordinative-crosslinked points (Figure S8).[12]

Table 1. Molecular weight (M_w) determined by SEC-MALS[a]

Entry	Ligand	Additive	M_w[b]/10^4 g mol^{-1}
1	ImCHP	—	52.9 (1.5)
2	ImCHP	Co(II)	55.4 (0.9)
3	ImCHP	Co(III)	87.3 (4.9)
4	ImCHP	β-CD	9.1 (0.3)
5	ImCHP	Co(III) and β-CD	99.5 (1.5)
6	ImP	—	2.8 (0.1)
7	ImP	Co(II)	2.8 (0.1)
8	ImP	Co(III)	6.1 (0.1)

[a]Concentration: [ImCHP], 4.7 mg mL^{-1}; [ImP], 3.8 mg mL^{-1}; [$CoCl_2$], 3.8 mM; [β-CD], 3.0 mM. The molar ratio of imidazole unit in polymer and cobalt ion is 10. The Co(II) was oxidized to Co(III) by the addition of 0.3% (w/w) H_2O_2 for 30 min. [b]The standard deviation of three independent measurements is in parenthesis.

In conclusion, pullulan- or CHP-embedding metal ligands were prepared to afford coordinative-crosslinking nanogels. The redox couple of Co(II) and Co(III) was utilized for construction of a redox-sensitive nanogel system. To the best of our knowledge, this is the first report of the preparation of coordinative-crosslinking nanogels with redox-sensitivity. This strategy of hybrid nanogels by self-assembly using hydrophobic interactions and coordinative interactions of polymers will be useful for the development of new nanobiomaterials.

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