Loading and Release of Charged Dyes Using Ultrathin Hydrogels

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The anionic dyes methyl orange (MO) and allura red (AR) were used as model drugs to assess the loading and release by layer-by-layer assembled ultrathin hydrogels prepared via the amide formation of poly(acrylic acid-*co*-*N*-isopropylacrylamide) with AAc contents of 5, 10, and 15 mol % plus poly(vinylamine hydrochloride). The amount of MO loaded was potentially controlled by changing the dye concentrations, film thickness, and AAc content of the copolymers. The release of AR was controlled by the NaCl concentration and pH. We conclude that the polymeric matrices of ultrathin hydrogels have great potential for the loading and release of charged drugs.

Introduction

Sustainable and controllable drug release enhances the effective treatment of disease, due to lower toxicity, tunable targeting, and improved patient convenience.[1,2] It is essential to chemically design polymeric matrices, which can readily embed various types and amounts of drugs, and then to release them in response to changes in external environment. It is therefore important to regulate not only the chemical structures of the matrices, but also the assembled structures of the component polymers at the macromolecular level.

Layer-by-layer (LbL) assembled polyelectrolyte multilayer films,[3] prepared by the alternate immersion of solid substrates into aqueous solutions containing oppositely charged polyelectrolytes, are potential matrices for drug loading and release. Researchers have previously reported the loading and release of drugs (sometimes model dyes), from assembled structures of multilayer films[4,5] and the regulation of these components by LbL assembly.[6−9] The LbL assembly of weak polyacids under suitable acidic conditions can generate acidic functional groups, and the subsequent immersion of these films into aqueous dye solutions at neutral pH values resulted in the loading of cationic dyes against the newly generated charges in the films.[4,5] Next, the reimmersion into an acidic solution or a suitable polymer solution released the dyes. The use of sulfonated cyclodextrins in the LbL assembly process produced films that incorporated dyes through host−guest chemistry.[6] The use of charged microgels as film components produced insulin release from the films.[7] Hydrolytically degradable LbL films also demonstrated tunable drug release.[8] Furthermore, drug-conjugated polyelectrolytes as prodrugs were used for the components of the films, and the reduction of cell viabilities was revealed.[9] Although the loading and release of drugs has been achieved using LbL assembled films, further analysis using other films with different chemical properties remains to be performed.

LbL assembled films prepared by the chemical cross-linking of copolymers with neutral and hydrophilic monomer units swell with water molecules in their aqueous phase, thus forming ultrathin hydrogels.[10−13] The swelling properties as well as the chemical compositions can be delicately modified by changing the component polymers used, as well as by changing the assembly conditions. Since the component polymers are chemically cross-linked, ultrathin hydrogels are stable even in concentrated ionic solutions,[10] although LbL assembled polyelectrolyte multilayer films conventionally prepared by weak polyacids were decomposed by ions.[14] In addition, small molecules such as ions and dyes are readily incorporated into swollen ultrathin hydrogels[13] through suitable interactions between functional groups remaining in the ultrathin hydrogels and the incorporating species. Accordingly, ultrathin hydrogels have great potential as drug matrices. Here, we report the tunable loading of charged dyes as model drugs into ultrathin hydrogels, and the release of dyes depending on the aqueous ionic strength and the pH values. LbL amide formation between poly(acrylic acid-*co*-*N*-isopropylacrylamide) [poly(AAc-*co*-NIPAAm)] with AAc contents of 5, 10, and 15 mol %, plus poly(vinylamine hydrochloride) (polyVAm) containing suitably cationic VAm units in ultrathin hydrogels resulted

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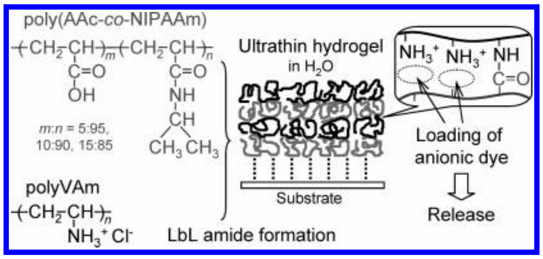

Figure 1. Schematic representation of this study.

in the electrostatic loading of anionic dyes. Accordingly, methyl orange (MO) with a single sulfonyl charge unit and allura red (AR) with double sulfonyl charge units were used for comparison. The schematic representation of this study is shown in Figure 1.

Experimental Section

Poly(AAc-*co*-NIPAAm)s were synthesized according to methods described in a previous study.[15] 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide hydrochloride (EDC) (>98.0%) was purchased from Wako (Japan), and was used without further purification. MO (Wako) and AR (Wako) were used without further purification.

The preparation of the ultrathin hydrogels was essentially the same as described in our previous study.[13] Briefly, a quartz substrate (35 × 9 × 1 mm) was immersed in an aqueous poly-(AAc-*co*-NIPAAm) solution (0.05 unitM) containing EDC (equimolar amounts to the total AAc units), in which the carboxyl group had been already activated for 5 min before immersion. The substrate was immersed for 15 min, rinsed gently with pure water, and then dried under N$_2$ gas. The substrate was immersed again into an aqueous polyVAm solution (0.05 unitM), and the same procedure was repeated. This sequential reaction cycle was repeated for a specific number of steps. Assembly was started with poly(AAc-*co*-NIPAAm)s. Although the substrate was not modified before assembly, the ultrathin hydrogels remained stable on the substrate. For the quantitative analysis of the amount assembled, a quartz crystal microbalance (QCM) with gold electrodes was used as the substrate.[10-13] The amount of polymer deposited, Δm (ng), was estimated by measuring the frequency shift of the QCM, ΔF (Hz), using Sauerbrey's equation[16] as follows:

$$-\Delta F = \frac{2F_0^{\ 2}}{A\sqrt{\rho_q \mu_q}} \times \Delta m$$

where F_o is the parent frequency of the QCM (9×10^6 Hz), A is the electrode area (0.159 cm^2), ρ_q is the density of the quartz (2.65 g cm^{-3}), and μ_q is the shear modulus (2.95×10^{11} dyn cm^{-2}). The frequency was detected in air after the assembled polymers were sufficiently dried under nitrogen gas, so as not to detect swollen water molecules, following the methods described in previous studies.[10-13]

For the loading of the dyes, the coated substrate was immersed into dye solutions at the specified concentrations for 15 min at 20 °C, rinsed with pure water, and then dried under nitrogen gas. Dye absorption was analyzed by ultraviolet−visible (UV−vis) spectrometry using a Jasco model V-550 at ambient temperature. Attenuated total reflection (ATR) spectra (Specrum One, Perkin-Elmer) were obtained using a refractive surface of the QCM gold electrode in air. The interferograms were coadded 50 times and Fourier transformed at a resolution of 4 cm^{-1}. For the release of dyes from the ultrathin hydrogels, the substrate coated with dye loaded ultrathin hydrogels was immersed into aqueous solutions with various NaCl concentrations and various pH values at 20 °C, and then the absorption of the aqueous phase was analyzed by UV−vis spectroscopy.

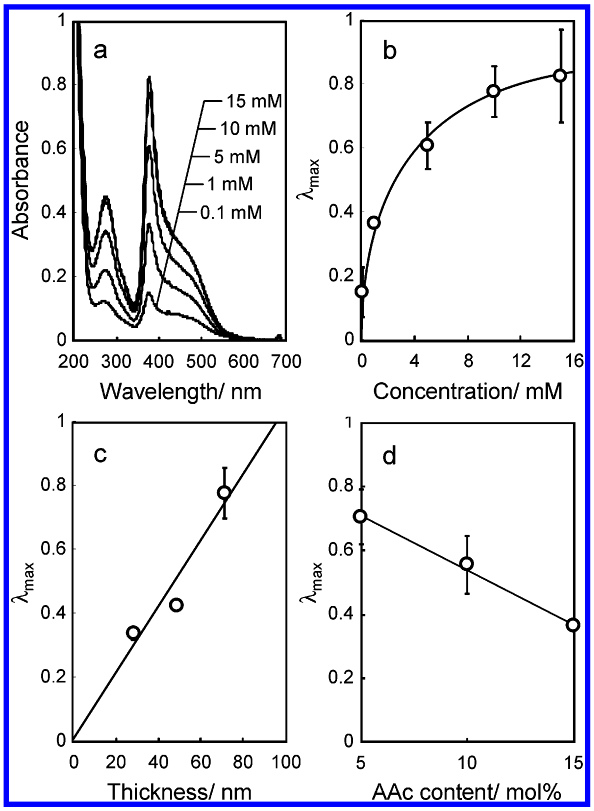

Figure 2. (a) UV−vis spectra of MO loaded into ultrathin hydrogels prepared by a 16-step assembly from poly(AAc-*co*-NIPAAm) with a 15 mol % AAc content and polyVAm at various MO concentrations, (b) the λ_{max} of the MO loaded into 16-step ultrathin hydrogels against the MO concentration, (c) the λ_{max} of MO loaded into 16-step ultrathin hydrogels at 10 mM MO against the film thickness, and (d) the λ_{max} of MO loaded into ultrathin hydrogels at 1 mM MO against the AAc content of poly(AAc-*co*-NIPAAm)s, in which 8, 12, and 16 steps were used to assemble copolymers with 5, 10, and 15 mol % AAc contents.

Results and Discussion

In a previous study,[13] anionic MO (instead of cationic dyes such as methylene blue) was preferentially incorporated into ultrathin hydrogels prepared from poly(AAc-*co*-NIPAAm)s and polyVAm, indicating that the hydrogels were suitable for the loading of anionic dyes. In other words, since almost all AAc units from the copolymers reacted with the VAm units of the polyVAm for LbL chemical reactions, only cationic VAm units remained in the ultrathin hydrogels. In the present study, we analyzed the controlled loading of MO into ultrathin hydrogels under various conditions.

The change in the concentration of MO regulates the amount of MO loaded. We observed two absorption peaks at 275 and 375 nm for MO after loading into 16-step assembled ultrathin hydrogels prepared from poly(AAc-*co*-NIPAAm) with a 15 mol % AAc content and polyVAm, as shown in Figure 2a. The maximum absorbance at 375 nm (λ_{max}) was largely blue-shifted as compared to the λ_{max} in aqueous solution (467 nm), suggesting that MO electrostatically interacted with the VAm units in the ultrathin hydrogels. ATR spectra of the MO-loaded film revealed the peak of symmetric vibration bands for sulfonyl groups of MO at 1119 cm^{-1}, while the peak of a MO powder was observed at 1112 cm^{-1}. This shift also suggest the electrostatic interaction of MO with the VAm unit. From the release analysis (see below), 62 nmol of MO per quartz substrate were loaded into the ultrathin hydrogel in 1 mM MO.

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The increase in the MO concentration resulted in an increase in the absorbance, with the same peak positions. The $\lambda_{\max}$ plotted against the MO concentration showed a saturation curve, and the amount of MO loaded was saturated at approximately 10 mM, as shown in Figure 2b. These observations indicated that the loading amount can be controlled by the MO concentration. From the saturation curve, the binding constant of MO to the ultrathin hydrogel was estimated by assuming the presence of a Langmuirian type adsorption as follows:

$$\frac{[\mathrm{MO}]}{\mathrm{Abs}} = \frac{1}{\mathrm{Abs}_{\max}}[\mathrm{MO}] + \frac{1}{\mathrm{Abs}_{\max}K}$$

where $\mathrm{Abs}_{\max}$ is the maximum absorbance and K is the binding constant. The data could be fitted to the equation with the coefficient of variation of 0.99, and the binding constant was estimated to be 8.0×10^2 M^{-1}.

The film thickness for assembly (8, 12, and 16 steps) can also change the loading amounts, as shown in Figure 2c. An increase in film thickness, which was estimated in air,[13] resulted in an almost linear increase in the amount loaded. These observations indicated that the amount of MO loaded can be regulated by the film thickness, and that the internal structure of ultrathin hydrogels are homogeneous even after the increased thickness. Furthermore, a change in the AAc content of poly(AAc-*co*-NIPAAm)s for assembly resulted in characteristic changes in the amounts loaded (note that the assembly step numbers were changed to obtain similar assembly amounts, 14.6 μg cm^{-2}). The amount loaded decreased with an increasing AAc content, as shown in Figure 2d. Since the assembly of poly(AAc-*co*-NIPAAm)s with a greater AAc content utilized greater amounts of VAm, the total amount of VAm units remaining in the ultrathin hydrogels should be decreased with increasing AAc content. This decrease in the VAm amount resulted in a decrease in the amounts of MO loaded. As a consequence, the MO concentrations, the film thickness of the assembly, and the AAc contents of the copolymers potentially regulated the amounts of MO loaded. We found that changes in these parameters readily changed the loading amounts.

Electrostatically loaded dyes can be released into ionic solutions (hindrance of electrostatic interactions) and at suitable pH (disappearance of the VAm charges). Although the release of MO was analyzed in ionic solutions, the release rates as well as the amount of MO released could not be controlled (all of the MO could be released even in the presence of 0.1 mM NaCl), possibly due to the fact that the interactions of MO with VAm were weakly responsive to the external environment. Although the release of dyes with a single charge unit were demonstrated using conventionally assembled polyelectrolyte multilayer films,[4,5] the interactions of MO with VAm in the water-swelling environment of the ultrathin hydrogels seemed to be weak. Therefore, we selected AR, an anionic dye with double charge units, to strengthen the interactions with the VAm units. In fact, AR was similarly loaded into the ultrathin hydrogels. A total of 130 nmol of AR per quartz substrate was loaded into ultrathin hydrogels prepared by poly(AAc-*co*-NIPAAm) with a 15 mol % AAc content and polyVAm in 1 mM AR. The loading amount of AR was greater than that of MO. Since AR has double charges, AR was more readily loaded to ultrathin hydrogels through electrostatic interactions. Since AR has double charges, the amounts of the VAm units interacting with AR were approximately 4 times greater than those interacting with MO (62 nmol) at the same dye concentration. Considering the data in Figure 2b, AR could interact with the VAm units that were not used for the interaction with MO. In other words, the free VAm units seemed to be remained in the case of MO loading even at higher MO concentrations.

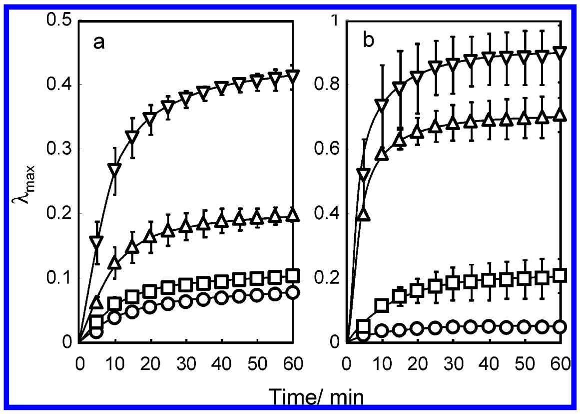

Figure 3. Release of AR loaded at 1 mM AR into ultrathin hydrogels prepared by 16-step assembly from poly(AAc-*co*-NIPAAm) with a 15 mol % AAc content and polyVAm, (a) against the NaCl concentration (circle, square, triangle, and reversed triangle indicate the release in water containing 1, 10, and 100 mM and 1 M NaCl, respectively) and (b) against the solution pH (circle, square, triangle, and reversed triangle indicate the release in water with pHs of 6, 8, 10, and 12, respectively).

Figure 3a shows the time dependence of the amounts of AR released (loaded at 1 mM) from the ultrathin hydrogel when the NaCl concentration was changed from 1 mM to 1 M (pH 5.6). For the initial 20 min, AR was rapidly released, and then the AR was gradually released. The release rates increased with increasing NaCl concentrations, and thus the concentration could control the release rates. In addition, sustainable release was also realized. Even after 60 min in 1 M NaCl, approximately 50% of the AR still remained in the ultrathin hydrogels. These observations suggest that drugs loaded into ultrathin hydrogels can be more slowly and continuously released under physiological ionic conditions. Release at suitable pH values is more dramatic. Although AR was not released under acidic pH conditions, almost all of the AR was rapidly released after 30 min at pH values 10, as shown in Figure 3b. Since polyVAm has a pK_a of 10,[17] these observations strongly suggest that the disappearance of the cationic VAm charges triggered the release of AR. In fact, the release profiles between 20 and 55 min could be fitted to Fick's second law[18,19] (the profiles until 20 min were not fitted due to the small number of data points), suggesting that the release was governed by classical diffusion of AR (see Supporting Information). In other words, AR was simply diffused from ultrathin hydrogels into aqueous phase once the interaction of AR with the VAm units was disappeared. Diffusion coefficient was not determined, because the film thickness might change during release. As a consequence, we found that the release of AR can be performed by changing the ionic strength and/or pH.

The ultrathin hydrogels prepared from poly(AAc-*co*-NIPAAm)s and polyVAm showed reversible change in permeabilities of ions below and above a lower critical solution temperature (LCST) of the polyNIPAAm units.[13]

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Although we preliminarily analyzed the release of dyes below and above a LCST, the LCST properties did not affect the release. Further delicate analysis is necessary to reveal the thermoresponsive release of small molecules from the ultrathin hydrogels.

Conclusions

We demonstrated that anionic dyes as model drugs were electrostatically loaded into ultrathin hydrogels prepared by LbL chemical reactions between poly(AAc-*co*-NIPAAm)s and polyVAm. The amounts loaded could be altered by changing the dye concentrations, film thickness, and AAc content of the copolymers. The sustainable release of loaded dyes was demonstrated for dyes with plural charges. The release was governed by the ionic strength and pH of the immersing solution. Ultrathin hydrogels have potential applications as controlled releasing matrices for charged drugs. Although we demonstrated a system for anionic dyes, the suitable selection of component polymers should result in anionically charged ultrathin hydrogels.[9,10] The tuning of delicate structures within these ultrathin hydrogels will further regulate loading and release of small molecules.

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Supporting Information Available: Text discussing and figures showing the fitted data of the release profiles of AR to Fick's second law. This material is available free of charge via the Internet at http://pubs.acs.org.

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