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Albumin-crosslinked alginate hydrogels as sustained drug release carrier

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Abstract

To take advantage of the drug-binding ability of albumin as a component of drug delivery system, we have prepared hydrogels consisting of alginic acid (AL) and recombinant human serum albumin (rHSA) by dehydrating condensation using N-hydroxysuccininimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. As rHSA content increased, the swelling ratio of the hydrogel decreased, indicating rHSA functioned as a crosslinker. In fact, trypsin treatment solubilized the hydrogel. Salicylic acid, which has high affinity for rHSA, was loaded most on the hydrogel of the highest rHSA content despite the lowest swelling ratio. Meanwhile, drugs with less affinity for HSA such as *o*-anisic acid and benzoic acid were preferably loaded on the hydrogel having the highest swelling ratio but the lowest HSA content. The release of salicylic acid from the hydrogel sustained longer than *o*-anisic acid and benzoic acid, reflecting the affinity of the drug for HSA. Furthermore, the hydrogel could carry much of positively charged dibucaine by the interaction with anionic alginic acid and showed highly sustained release. Since the safety of AL and rHSA in medical use is guaranteed, rHSA-crosslinked AL hydrogel is expected to use as a sustained drug release carrier for drugs having affinity for HSA and those with cationic charge.

Keywords: Albumin; Biodegradable hydrogel; Drug delivery; Sustained release; Crosslinking with proteins; Alginic acid

1. Introduction

It is important to supply drugs to the patient in a controlled rate enabling the appropriate concentration of drugs and prolonged effectiveness. Crosslinked hydrogel networks have been investigated as controlled drug delivery systems, taking advantage of their functions to up-take and release drugs. Drugs confined in a hydrogel polymer network are released to a milieu in a rate controlled by the swelling behavior of hydrogels, the pore size of a polymer network, the affinity between drugs and hydrogel-constituting polymers, and hydrogel degradation *in vivo*. Hydrogels consisting of ionic polymers such as hydroxyethyl methacrylate and methacrylic acid have been extensively applied to the drug delivery vehicle due to the ionic interaction between polymers and counterionic drugs and the swelling behavior varying with pH and buffer conditions. [1–8] However, these hydrogels are not degradable by either hydrolytic or enzymatic mechanisms, which limit their potentials as biodegradable drug carriers.

Recently, we prepared a hydrogel in which bovine serum albumin (BSA) was used as nodes of three-dimensional hydrogel network by linking BSA with poly(acryl amide) chains, focusing on the binding ability of albumin to various substances such as amino acids and drugs. [9] Usage of albumin as a crosslinking molecule has advantages in preventing albumin from leakage out of a hydrogel, which may occur if albumin is merely embedded in a hydrogel. In addition, the hydrogel was shown to become solubilized by proteolytic digestion of BSA, indicating that the hydrogel would be cleared in the body, which may decrease harmful body response caused by semipermanent residual of a hydrogel in the body. [10] BSA-crosslinked poly(acryl amide) hydrogel was demonstrated to show sustained release for drugs having affinity for albumin, that is, the drugs with high affinity to BSA was loaded in a higher amount and was released more slowly than those having less affinity to BSA. However, acryl amide monomer is likely to be harmful if it remained in a hydrogel and BSA possibly causes immunogenic adverse effects for human. Therefore, we have replaced BSA and acryl amide with recombinant human serum albumin (rHSA) [11] and alginic acid (AL), respectively. Since

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HSA and AL have been granted as pharmaceuticals or foods additive, respectively, the safety of resultant hydrogel is guaranteed. Since AL is anionic polysaccharide, the resultant hydrogel is expected to bind to both drugs with cationic charge and drugs having affinity to albumin. The present paper describes the preparation of hydrogels consisting of rHSA and AL and their *in vitro* evaluation as a drug carrier.

2. Materials and methods

2.1. Materials

Recombinant human serum albumin (rHSA) was kindly provided from Mitsubishi Pharma Corporation (Osaka). Sodium alginate 80–120 cP (AL), ethylenediamine (EDA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), and salicylic acid were purchased from Wako Pure Chemical Industries (Osaka). Sodium benzoate, *o*-anisic acid, and dibucaine hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of rHSA-crosslinked alginate hydrogel (HSA-AL)

AL was dissolved in 0.05 M 2-morpholinoethanesulfonic acid (MES) buffer (pH 5.6) and cooled to 4 °C on ice. EDC and NHS were added to AL solution to activate carboxylic acid groups of AL. After 20-minutes preactivation at 4 °C, rHSA was added to the reaction mixture with gentle shaking. A combined weight of rHSA and AL was 600 mg and the volume of the mixture was adjusted to 12 ml with MES buffer. The amount of rHSA and AL actually used in hydrogel preparation is shown in Table 1. Then the reaction mixture was cast to perfluoro alkoxy fluoroplastics (PFA) dish (internal diameter: 50 mm) on which glass plate was placed to avoid drying and stood for 1 day at 4 °C. The resultant hydrogel was cut to a cylindrical shape by cork borer (diameter: 8.5 mm). Thus obtained hydrogel disk was washed in 50 g/l of Na_2HPO_4 solution and distilled water at 4 °C for 3 days, respectively. The hydrogel disk was stored in distilled water at 4 °C.

The EDA-crosslinked AL hydrogels (EDA-AL) were prepared as a control in a similar manner to the HSA-AL. The compositions of the EDA-AL are also shown in Table 1.

Table 1
Preparation of hydrogels and their swelling ratios

Hydrogel	HSA or EDA (mg)	AL (mg)	AL-COOH/HSA or EDA [a] (mol/mol)	Swelling ratio [b] (*W*s/*W*d) [c]
HSA-AL (11:1)	550	50	30	44±7
HSA-AL (5:1)	500	100	67	56±7
HSA-AL (1:1)	300	300	330	81±4
HSA-AL (1:5)	100	500	1670	137±14
EDA-AL (1:33)	18	582	10	41±3
EDA-AL (1:1000)	0.6	599	320	122±18

[a] The mol ratio of monosaccharide in AL to HSA or EDA.
[b] Each hydrogel came to equilibrium in distilled water.
[c] *W*s and *W*d are the weights of the swollen and dried hydrogels, respectively.

2.3. Swelling of hydrogels

The hydrogel was left in distilled water until reaching to a constant size. Then, the weight of the hydrogel was measured after the moisture on the surface was removed with a filter paper. The hydrogel was also weighed after drying for a week in a desiccator. The swelling ratios (*SRs*) of hydrogels were calculated using $SR=(Ws-Wd)/Wd$, where *W*s and *W*d are the weight of the swollen and dried hydrogels, respectively.

2.4. Trypsin treatment of HSA-AL and EDA-AL hydrogels

The hydrogels swollen in distilled water were immersed in Dulbecco's phosphate buffered saline (-) (DPBS) containing 0.25%(*w/v*) of trypsin at 37 °C for 2 weeks, changing trypsin solution everyday. The change in hydrogel appearance was recorded with photographs.

2.5. Adsorption of drugs to rHSA

Twenty milliliters of rHSA aqueous solution (5 mg/ml) was placed in a seamless cellulose tubing (molecular cutoff of 12,000–16,000; Wako Pure Chemical Industries) and immersed in 380 ml of 0.53 mM drug solution at room temperature for 3 days, by which the drug concentration reached to a constant value. Then, the amount of the drug adsorbed to one rHSA molecule was calculated from the drug concentration in the outer solution.

2.6. Drug loading on a hydrogel

Each drug was dissolved in distilled water to prepare 2 mM solution. Hydrogels swollen in distilled water were soaked in each drug solution at room temperature for 3 days. Then, the drug-loaded hydrogels were dried for 1 week in a desiccator.

2.7. Drug release from hydrogels

The dried drug-loaded hydrogels (10 mg) were immersed in 50 ml of DPBS at room temperature. The medium was periodically replaced, and the amount of drug released into the medium was quantified by measuring the absorbance of the drug at 296 nm (salicylic acid and *o*-anisic acid in 1 M HCl), 224 nm (sodium benzoate), or 246 nm (dibucaine hydrochloride in 1 M HCl). After 2 weeks, the hydrogels were placed in DPBS at 60 °C for 24 h to complete the drug release. UV spectra were collected using a U-2010 spectrophotometer (Hitachi High Technologies, Tokyo).

3. Results and discussion

3.1. Hydrogel preparation

Hydrogels consisting of rHSA and AL were prepared according to the procedure shown in Fig. 1. AL was firstly converted its activated ester by dehydrating condensation with NHS by the aid of water-soluble carbodiimide, EDC. Subsequently,