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Salt effects on the hydration behavior of zwitterionic poly(sulfobetaine methacrylate) aqueous solutions

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ABSTRACT

Hydration behavior of a biomaterial is thought to be closely related to its bio/blood compatibility and subsequent biological responses. Poly(sulfobetaine methacrylate) (pSBMA) is one of the biomimetic materials that exhibits excellent biocompatibility. It has been reported that the zwitterionic pSBMA exhibits "antipolyelectrolyte" behavior, which changes polymer conformations and antifouling properties, in solutions containing salt ions. In this study, we identified water hydrated states of pSBMA by thermal analysis of DSC in various salt concentrations. With the addition of miniscule salts, ions are attracted to the zwitterionic groups and the hydrated states are significantly changed. The attracted ions promote the incorporation of water molecules into polymer chains and enhance the solubility of pSBMA. Nevertheless, when large amounts of salts are added into the system, the excess ions tend to bind with free water molecules, which may impose an osmotic pressure on the hydrated pSBMA molecules and causes the shrinkage of the molecules. The present results demonstrated that salt has a significant effect on pSBMA–water interactions, which may affect the biocompatibility of the material.

1. Introduction

The bio-/blood compatibility is one of the most important characteristics of a biomaterial for its applications in medical field. When a polymer comes into contact with blood, spontaneous adsorption of plasma proteins often induces the subsequent inflammatory responses and thrombosis formation [1]. Many attempts have been proposed trying to clarify the protein-resistant mechanism [2]. It is generally believed that the non-biofouling ability of a material is strongly correlated with the hydration layer vicinal to the material surface. A tightly bound water layer may form a physical and energetic barrier to prevent the possible protein adsorption [3]. Understanding the water structure on material surfaces is the key to developing biocompatible materials [4].

Various techniques, such as viscoelastic measurements, thermal analysis, infrared (IR) and Raman spectroscopy, have been applied to examine the water behavior on polymers [5–7]. Among them, differential scanning calorimetric (DSC) analysis is one of the advantageous techniques that can estimate the hydration properties of materials quantitatively with a broad range of water content. From the thermal data reported for various hydrated polymers, the water structures have been categorized into three groups: nonfreezing, freezing bound and free water [8]. The water that does not crystallize even at −100 °C, is defined as nonfreezing water. These water molecules bind with polymers so strongly that no thermodynamic first-order phase transition could be detected in the thermal cycle. The freezing bound water, also called "intermediate water", is a kind of water that binds to material surfaces and yet still reveals thermodynamic first-order phase transition. The transition temperature of freezing bound water (T_{fb}) is usually substantially lower than the transition temperature of bulk water (T_{bulk}). The cold crystallization peak sometimes observed in a heating run of a DSC test, which has been attributed to the slow packing of the freezing bound water [9], can be used as an auxiliary to distinguish the existence of the freezing bound water. Free water is the one interacting weakly with polymers and crystallizing at temperature between T_{fb} and T_{bulk}. Supplementary Fig. 1 illustrates the classification of various water structures of a hydrated polymer system.

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A large number of biomaterials, including hyaluronan, poly(ethylene glycol) (PEG), poly(2-hydroxyethyl methacrylate), poly(2-methoxyethyl acrylate), etc., have been investigated by DSC to interpret the water–polymer interactions [10,11]. From previous reports, freezing bound water is mainly observed in

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biocompatible synthetic and natural polymers, such as PEG, PMEA and polysaccharides. Tanaka et al. speculated that the blood compatibility of a polymer is related to the presence of freezing bound water [12]. On the other hand, Morisaku et al. found that the biocompatible pMPC hydrogel had larger nonfreezing water than the p-Me(EG)$_n$MA hydrogel [13]. These findings suggested that the hydrated states of a material characterized by DSC have the potential to becoming an index for blood compatibility.

Many biomimetic polymers have been studied in order to develop novel biomaterials. Among them, poly (sulfobetaine methacrylate) (pSBMA), a zwitterionic sulfobetaine polymer containing both positive and negative charges on its backbone (Fig. 1), has been found to exhibit ultralow biofouling properties and is able to bind water molecules more strongly via electrostatically induced hydration instead of hydrogen bonding [2]. Their functionalities and applications have become an interesting subject in recent years. However, it is also reported that the zwitterionic polymer exhibits "antipolyelectrolyte" behavior in solutions containing salt ions, which may change polymer chain conformations and affects its protein resistant properties [14]. Studies showed that zwitterionic surfaces adsorb much more proteins in a low ionic strength solution ($<$0.1 M) compared with a high ionic strength (0.1–0.3 M) solution [15]. It is considered that the electric field of the dipole moment on zwitterionic groups could be partially screened by the counter ions in ionic solution, resulting in better antifouling performance [16]. On this account, it seems apparent the small amount of ions existing in biological systems would affect the interactions between water, polymers and proteins. For suitable design of blood compatible materials in practical application, it is necessary to deeply understand the effects of ion solute (salt) on the water structure of hydrated zwitterionic polymers.

In this study, we paid particular attention on the role of ions playing in the pSBMA-water system. We investigated the structural change of water in pSBMA with different ionic concentration by using DSC to clarify the hydration states of pSBMA.

2. Materials and methods

2.1. Materials

The monomer [2-(methacryloyloxy)ethyl] dimethyl-(3-sulfopropyl)ammonium hydroxide (SBMA, 97%) was purchased from Sigma–Aldrich (Milwaukee, WI). The initiators and solvent, sodium metabisulfite (SBS, 99.8%), ammonium persulfate (APS, 98.8%), and ethanol (99.9%), were purchased from J.T. Baker (Netherlands). All solvents and chemicals were used as received without further purification. Deionized water used in these experiments was purified by a Millipore water purification system with a minimum resistivity of 18.2 MΩ cm. Sodium chloride was purchased from Merck KGaA (purity $\geq$ 99.5%, $<$0.5% loss on drying (105 °C, 2 h), Darmstadt, Germany).

2.2. Polymerization

Poly(sulfobetaine methacrylate) (pSBMA) was prepared by free radical polymerization initiated by ammonium persulfate (APS) using a protocol adapted from a previous study [17]. In short, the polymerization of pSBMA was carried out in an aqueous ethanol solution under static conditions at room temperature for 24 h. After polymerization, pSBMA was observed to precipitate from the resulting reaction solution. The precipitates were dissolved in hot water and purified in ethanol solution three times. Finally, the polymer solutions were dried in a freeze-dryer at −45 °C to yield a white powder.

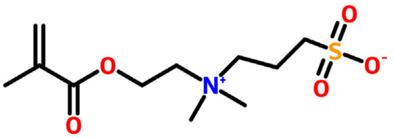

Fig. 1. Monomer structure of poly(sulfobetaine methacrylate) (pSBMA).

2.3. Characterization

The chemical structure of the synthesized pSBMA was characterized by FTIR spectroscopy and by ^{1}H NMR spectrometry using D_2O as solvent. ^{1}H NMR (400 MHz, D_2O), δ (ppm) = 1.97 ($CH_2C(CH_3)COO$), 2.26 ($CH_2CH_2CH_2SO_3$), 2.96 ($CH_2CH_2SO_3$), 3.21 ($CH_2N^+(CH_3)_2CH_2$), 3.57–3.60 ($CH_2CH_2N^+(CH_3)_2$), 3.79 ($CH_2N^+(CH_3)_2CH_2CH_2O$), 4.48 (CH_2CH_2OCO). According to the characteristic peaks of pSBMA segments and the disappearance of vinyl double bonds at δ of 5.7 and 6.1 ppm, we confirmed the successful polymerization of the SBMA monomer [18]. The average molecular weight of pSBMA (M_v = 308,000 dalton) was estimated from the Mark–Houwink equation $[\eta] = 2.06 \times 10^{-3}\ M^{0.4071}$ ($[\eta]$ in dl/g) in 0.2 M aqueous NaCl solution at 21 ± 0.2 °C [17].

2.4. DSC measurement

Proportion of different types of water present in hydrated pSBMA was determined by the phase transition of water from thermal analysis, using a Perkin Elmer differential scanning calorimeter (Pyris Diamond DSC) equipped with a cooling apparatus. Before the DSC measurement, polymers and salts were dried in a freeze dryer for one day to eliminate the intrinsic water in the polymer matrix. About 5–10 mg pSBMA samples with known water content were injected into and hermetically sealed in aluminum pans to avoid possible water evaporation. Aluminum sealed pans were weighed by a Mettler Toledo microbalance (AX205) with precision $\pm 1.0 \times 10^{-5}$ g Samples were kept at room temperature overnight for equilibrium and weighed again in order to confirm no mass loss. The samples were cooled from 25 °C to −100 °C to freeze the solution and then heated from −100 °C to 25 °C with a 10 °C/min scan rate, avoiding the lag of response caused by a faster heating rate. Samples for the second run were checked and were showed no difference with the first measurement. Samples were weighed after tests to further confirm no mass change.

Using the assumption that the melting enthalpy of freezing bound water/free water are the same as that of bulk water (333.5 J/g) [19], we are able to estimate the amount of different types of water quantitatively through integrating the endotherms and exotherms shown in the DSC data. In order to study the water-polymer-ion interactions, we defined the following relative water contents:

n_{H_2O}/n_{SBMA} (mol/mol) = numerical ratio of water molecules to SBMA unit.

n_{H_2O}/n_{NaCl} (mol/mol) = numerical ratio of water molecules to NaCl molecules.

The amount of water corresponding to the four hydration states: nonfreezing, freezing bound, free and bulk water are denoted as n_{nf}, n_{fb}, n_{free}, and n_{bulk}, respectively. The total amount of water added in the sample and freezable water obtained from the heating run of the DSC curve are represented by n_{added} and $n_{freezable}$, respectively. Here,

$$n_{freezable} = n_{fb} + n_{free} + n_{bulk}$$

$$n_{nf} = n_{added} - n_{freezable}$$