Polypeptide for anhydrous proton conductor

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ABSTRACT

The anhydrous proton conductor consisting of a polypeptide was prepared by the mixing of polyglutamic acid (poly(Glu)) and polyhistidine (poly(His)), which were synthesized by the polymerization of an amino acid with diphenylphosphoryl azide (DPPA). These polypeptide hybrid materials interacted between the carboxyl group of poly(Glu) and the imidazole group of poly(His) and produced the acid-base structure. As a result, these polypeptide hybrid materials showed a thermal stability at intermediate temperatures ($\leq 200\,^\circ$C). Additionally, these hybrid materials indicated the proton conductivity of 1.4×10^{-4} S cm^{-1} at 180 $^\circ$C under anhydrous conditions. Furthermore, an activation energy of the proton conduction under anhydrous condition was 0.52–0.63 eV, one order higher than that of the customary humidified perfluorinated membranes. These results suggested that the proton transfer of hybrid material occurred by the anhydrous proton conductive mechanism. Therefore, the polypeptide hybrid material may have the potential to be utilized as a novel proton conductor under anhydrous condition.

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1. Introduction

Polymer electrolyte membrane fuel cell (PEFC) is one of the clean energy conversion systems. Therefore, PEFC at low temperatures ($\leq 80\,^\circ$C) with customary perfluorinated sulfonic acid membranes, such as Nafion®, have been developed for mobile, satellite, or stationary applications. However, the high cost of electrolyte membranes and the complicated water management are major obstacles for practical applications of PEFC. Furthermore, at low temperature, carbon monoxide (CO), which is contained in the hydrogen gas reformed by hydrocarbons such as natural gas, gasoline, or alcohol, absorbs on Pt electrode and causes the tolerance of electrode. As a result, the energy efficiency decreases with the generation time. On the other hand, the operation of the PEFC at intermediate temperature (100–200 $^\circ$C) has been considered to provide many advantages, such as an improved CO tolerance of the Pt electrode, higher energy efficiency, simplified heat management, and co-generations [1–3]. However, since the proton conduction of the customary electrolyte membranes at low temperatures ($\leq 80\,^\circ$C) is based on the existence of mobile water molecules in the membrane, the proton conductivity at intermediate temperature abruptly decreases due to the evaporation of water contents [1–5]. Therefore, under an anhydrous or low

humidity conditions, a non-vehicular mechanism, in which only the protons are mobile from site to site without diffusible water molecules (Grotthuss mechanism), is important for future PEFC technology [6]. On the other hand, the production cost of customary perfluorinated membranes is extremely high. Therefore, we attempted to the utilization of a biopolymer as an anhydrous proton conductor [4]. Biopolymers are polymers produced by living organisms. The representative biopolymers are polysaccharides, proteins, and polynucleotides. We have reported an anhydrous proton conductor consisting of a polysaccharide or polynucleotide, such as chitin-chitosan [7,8], alginic acid [9], as a non-expensive electrolyte material. In addition, we prepared the anhydrous proton conductor consisting of DNA which can be easily purified from salmon milts [10]. Since the DNA-containing materials, such as salmon milts, are discarded as industrial wastes around world, these proton conductive materials are low cost. In addition, other groups also have reported a proton conductor consisting of a polysaccharide, such as carrageenan [11], starch [12], and gum arabica [13,14]. These biopolymers are low cost, nonhazardous, and biodegradable [15,16]. Additionally, many biopolymers have been discarded as industrial waste around the world. Therefore, the development of a proton conductor consisting of a biopolymer is an environmentally benign concept.

The non-vehicular mechanism without using diffusible water molecules has been reported for a composite material consisting of an acidic polymer and heterocyclic molecules, such as imidazole and benzimidazole [6]. In these composite materials, the basic heterocyclic molecules accept the free proton from the acidic polymer. As a result, the anhydrous proton transfers occur from the

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protonated heterocyclic molecule to the non-protonated hetero-cyclic molecule. Namely, the protonated and non-protonated heterocyclic molecules can play a role as a proton donor and acceptor, respectively. Therefore, we prepared an acid-base composite material consisting of a biopolymer, such as the chitin phosphate-imidazole composite [7], alginic acid-imidazole composite [9], and DNA-imidazole composite [10], and measured proton conduction under anhydrous condition. As a result, these composite materials showed high anhydrous proton conductivity on the order of 10^{-3} S cm^{-1}. However, although these investigations of an anhydrous proton conductive biopolymer have been demonstrated using a polysaccharide and polynucleotide, such as chitin-chitosan, alginic acid, or DNA, the anhydrous proton conductions of a protein or polypeptide have not been reported. Therefore, we attempted to use a synthesized polypeptide as the anhydrous proton conductor. Especially, polypeptides, which consisted of an amino acid, have various functional groups, such as a carboxyl group and imidazole group, and these functional groups might play a role as a proton donor and acceptor.

In this study, we synthesized polyglutamic acid (poly(Glu)) and polyhistidine (poly(His)) by the polymerization of L-glutamic acid derivative and L-histidine with diphenylphosphoryl azide (DPPA), respectively. The polypeptide hybrid material was prepared by mixing poly(Glu) and poly(His). The poly(Glu)-poly(His) hybrid material formed the acid-base structure through an electrostatic interaction and showed a thermal stability at intermediate temperatures. Furthermore, the poly(Glu)-poly(His) hybrid material indicated the proton conductivity of 1.4×10^{-4} S cm^{-1} at 180 °C under anhydrous conditions.

2. Experimental sections

2.1. Material

L-glutamic acid γ-benzyl ester Glu(OBzl), L-histidine (His), diphenylphosphoryl azide (DPPA), thioanisole, trifluoroacetic acid (TFA), *m*-cresol, and triethylamine (TEA) were purchased from Wako Pure Chemical Industries, Ltd. Osaka, Japan, Peptide Institute, Inc. Osaka, Japan, or Nacalai Tesque, Inc. Kyoto, Japan. Solvents were used an analytical grade in all the experiments described. Ultra-pure water (Merck KGaA, Darmstadt, Germany) was used in this experiment.

2.2. Synthesis of poly(Glu)

The molecular structure of poly(Glu) was shown in Scheme 1. The poly(Glu(OBzl)) was synthesized by a reported procedure [17]. Glu(OBzl) (1 g, 4.2 mmol) was dissolved in DMSO (2 ml). DPPA (1.06 ml, 8.4 mmol) and TEA (1.30 ml, 9.3 mmol) was added to the solution and stirred at room temperature for 48 hours. Water (20 ml) was added to the reaction mixture, the solid compound

was filtered off and washed with methanol (10 ml × 3 times) and diethyl ether (10 ml × 3 times), and then this compound was freeze-dried. The poly(Glu(OBzl)) was deprotected using TFA-thioanisole-*m*-cresol-H$_2$O (3.2 ml, 0.2 ml, 0.2 ml, 0.4 ml) at 0 °C for 3 hours [18]. This solution was freeze-dried, this compounds was washed methanol (10 ml × 3 times) and water (10 ml × 5 times), and then freeze-dried once more. The identification of the synthesized poly(Glu) was demonstrated by ^{1}H and ^{13}C NMR spectrum using a Gemini-300 (Agilent Technologies, Inc. Santa Clara, CA) and IR spectrum using a FT-IR 8400 Fourier transform infrared spectrometer (Shimadzu Corp., Kyoto, Japan). Additionally, the molecular weight of poly(Glu) was estimated by the gel permeation chromatography (GPC) using Asahipak® GF-510HQ column (Show Denko K.K., Tokyo, Japan) with the polystyrene standard (Agilent Technologies, Inc. Santa Clara CA) [19,20]. The eluent was used *N,N*-dimethylformamide (DMF). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 2.0 (2H,–γH_2–), 2.5 (2H,–βH_2–), 4.3 (1H,–αH–). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 25.3 (β), 30.0 (γ), 65.5 (α), 171 (C = O), 175 (COOH). The signals in the ^{1}H and ^{13}C NMR spectra of the synthesized poly(Glu) were consistent with the reported spectra [20].

2.3. Synthesis of poly(His)

The molecular structure of poly(His) was shown in Scheme 1. The poly(His) was synthesized by a reported procedure [17,21]. L-His (1 g, 6.4 mmol) was dissolved in DMSO (2 ml). DPPA (1.80 ml, 8.4 mmol) and TEA (2.06 ml, 15 mmol) was added to the solution and stirred at room temperature for 48 hours. Water (20 ml) was added to the reaction mixture, the solid compound was filtered off and washed with methanol (10 ml × 3 times) and diethyl ether (10 ml × 3 times), and then this compound was freeze-dried. The identification of the synthesized poly(His) was demonstrated by ^{1}H and ^{13}C NMR spectrum and IR spectrum. The molecular weight of poly(His) was estimated from GPC with polystyrene standard. The eluent was used DMF. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 3.0 (2H,–βH_2–), 4.5 (1H,–αH–), 7.0 (1H, C = CH-N), 7.9 (1H, N = CH-N). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 30.0 (β), 65.0 (α), 127 (C4), 128 (C5), 136 (C2), 172 (C = O). The signals in the NMR spectra of the synthesized poly(His) were consistent with the reported spectra [21].

2.4. Preparation and characterization of poly(Glu)-poly(His) hybrid material

The poly(Glu)-poly(His) hybrid material was prepared by mixing poly(Glu) and poly(His) in an agate mortar. The mixing ratio (R) of poly(Glu)-poly(His) hybrid material was determined by (weight of poly(His))/(weight of poly(Glu)). In this study, the R values of poly(Glu)-poly(His) hybrid material were 0–50.

The IR spectrum of hybrid material was demonstrated by the attenuated total reflection (ATR) method. The resolution of spectrum was 4 cm^{-1}. The thermal stability of the poly(Glu)-poly(His) hybrid material was analyzed by thermogravimetric - differential thermal analysis (TG-DTA) (DTG-60, Shimadzu Corp.). The TG-DTA measurement was carried out at a heating rate of 10 °C min^{-1} under a dry-nitrogen flow. Sample weights of TG-DTA measurements were normalized at 1 mg.

2.5. Proton conductive measurement of poly(Glu)-poly(His) hybrid material

Before the proton conductive measurements, the poly(Glu)-poly(His) hybrid materials were dried at 180 °C under dry-nitrogen flow conditions in stainless steel vessel for 3 hours to evaporate the water from proton conductive samples. The temperature of dried
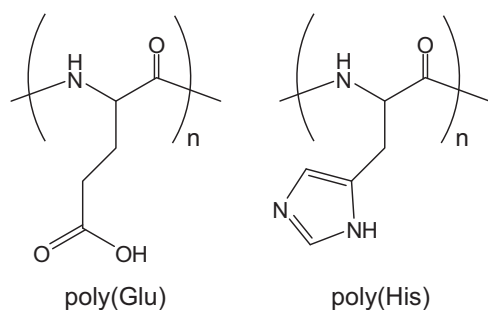
Scheme 1. Molecular structure of poly(His) and poly(Glu).